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Cortical Neuroplasticity and Cognition in Extreme Environments

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For My Little One

"Aujourd'hui nous allons devoir explorer Mars, des océans, l'univers, mais nous devons surtout explorer de ce que nous sommes."

Today, we explore Mars, the oceans, the universe, but above all,
we will have to explore who we are.

Christian Clot, French-Swiss Explorer

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List of Abbreviations

BDC	Baseline Data Collection
BOLD	Blood-oxygen-level Dependent
BrdU	Bromodeoxyuridine
CA3	Cornu Ammonis 3
CNES	Centre national d'étude spatiales *(French Space Agency)*
CPT	Continous Performance Task
CSF	Cerebrospinal Fluid
CTRL	Control Group
DLR	Deutsches Zentrum für Luft- und Raumfahrt *(German Aerospace Center)*
DRKS	Deutsches Register Klinischer Studien *(German Clinical Trials Register)*
EEG	Electroencephalography
EPI	Echo Planar Imaging
ERP	Event-related Potential
ESA	European Space Agency
FL	Flight Level
fMRI	Functional Magnetic Resonance Imaging
GM	Gray Matter
HDBR	Head-down tilt Bed Rest
HDT	Head-down tilt
IAPS	International Affective Picture System
ISS	International Space Station
kt IAS	Indicated Air Speed in Knots
LPP	Late Positive Potential
MCI	Mild Cognitive Impairment
MEDES	Institute for Space Medicine and Physiology
MRI	Magnetic Resonance Imaging
NASA	National Aeronautic Space Administration
PCG	Posterior Cingulate Gyrus
PET	Positron Emission Tomography
POMS	Profile of Mood States
PVT	Psychomotor Vigilance Test
ROI	Region of Interest

RSL	Reactive Jumps in a Sledge Jump System as a Countermeasure during Long-term Bed Rest *(study title)*
RT	Reaction Time
SANS	Spaceflight Associated Neuro-ocular Syndrome
SPM	Statistical Parametric Mapping
STAI	State-Trait Anxiety Inventory
TRAIN	Training Group
VR	Virtual Reality
WM	White Matter

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Abstract

Space is one of the most extreme environments for human beings, yet space faring nations strive for deep space exploration and human settlement on Moon and Mars. These missions expose crews to various physiological and psychological stressors such as radiation, altered gravity conditions, social isolation and confinement, and reduced physical activity - stressors that are also known to alter neuroplasticity and cognitive functions. To ensure missions' success and safety, it is important to know how the brain adapts in response to these stressors and to understand the associated neurobehavioral risks.

As part of this dissertation, the impact of three of those stressors on brain function was examined through two different spaceflight analog models, i.e., *Head-down tilt Bed Rest* and *Parabolic Flight*.

The effects of *Physical Inactivity* in form of long-term bed rest on episodic memory and its neural correlates by means of functional magnetic resonance imaging were the focus of *Research Paper I*. The impact of long-term head-down tilt bed rest, which is also characterized as a *semi-isolated*, *confined* and *sensory deprived environment*, on affective processing and its underlying electrocortical activity was investigated in *Research Paper II*. In *Research Paper III*, the impact of short bouts of *Microgravity* on attentional processes during a parabolic flight was examined.

First, the results showed that long-term immobilization of two months of Head-down tilt Bed Rest (HDBR) altered brain activity during memory encoding and retrieval in the left hippocampus and parahippocampal gyrus. These changes could be largely counteracted by a high-intensity training intervention that was performed during the immobilization period. Second, bed rest associated social isolation and confinement evoked reduced Event Related Potential (ERP) amplitudes in participants while looking at highly arousing photographs. This emotional blunting was observed predominantly in centroparietal regions and did not occur in a control group. Source localization confirmed a lower electrocortical activity in the posterior cingulate gyrus, insula, and precuneus in the bed rest group for pleasant and unpleasant, but not for neutral photographs. Third, attentional performance during a Continuous Performance Task (CPT) was impaired by weightlessness. There was additional evidence that attentional performance was also influenced by participants' emotional states.

The results provide evidence of the adverse neurobehavioral adaptations brought about by these spaceflight associated stressors. The results go beyond applications of space medicine and provide further insight into the adaptational processes of the brain in response to physical inactivity, confinement, and vestibular deficiency.

Zusammenfassung

Der Weltraum ist eine für Menschen unnatürliche Umgebung, dennoch streben die nationalen Raumfahrtagenturen vermehrt Langzeitmissionen und eine Kolonisierung von Mond und Mars an. Bei diesen (Langzeit-)Aufenthalten ist der Mensch verschiedenen physiologischen und psychologischen Stressoren ausgesetzt, die auch funktionelle und strukturelle Adaptionsprozesse in unserem Gehirn verursachen können. Neben den veränderten Gravitationsbedingungen und kosmischer Strahlung, stellen auch eingeschränkte körperliche Aktivität und soziale Isolation auf einem engen Raum ein potenzielles Risiko dar. Um die Sicherheit, aber auch den Erfolg solcher Missionen zu gewährleisten und um mögliche gesundheitliche Risiken vorherzusagen, ist es entscheidend, die Einflüsse von Langzeitaufenthalten im Weltraum auf kognitive Ressourcen und ihre neuronalen Korrelate zu erfassen.

In der vorliegenden Dissertation wurden drei dieser Stressoren und deren Auswirkungen auf die Hirnfunktion in drei separaten Studien näher untersucht. Dafür wurden zwei verschiedene Weltraumanalogmodelle genutzt – eine Bettruhestudie in Kopftieflage sowie ein Parabelflug.

In dem *ersten Forschungsartikel* wurden die Auswirkungen von *körperlicher Inaktivität* in Form von Langzeitbettruhe auf die episodische Gedächtnisbildung und deren neuronale Korrelate mittels funktioneller Magnetresonanztomographie untersucht. Die Auswirkungen auf die affektive Verarbeitung und die elektrokortikale Aktivität einer solchen Langzeitimmobilisierung, die zusätzlich auch durch eine *sensorische Deprivation*, *Beengtheit* und einer *Semi-Isolation* der Teilnehmer gekennzeichnet waren, standen im Mittelpunkt des *zweiten Forschungsartikels*. In dem *dritten Forschungsartikel* wurden die Auswirkungen von *Mikrogravität* auf Aufmerksamkeitsprozesse während eines Parabelflugs untersucht.

Die Ergebnisse zeigten, dass eine langfristige Immobilisierung die Hirnaktivität während der Gedächtnisenkodierung und des Abrufs im linken Hippocampus und im parahippocampalen Gyrus veränderte. Diesen Veränderungen konnte durch ein hochintensives Training, das während der Bettruhe durchgeführt wurde, entgegengesteuert werden. Zusätzlich verursachte die mit Bettruhe einhergehende soziale Isolation und Beengtheit eine Reduktion der Amplituden der ereigniskorrelierten Potenziale (EKP) beim Betrachten von hocherregenden Bildern. Diese emotionale „Abstumpfung“ wurde vor allem in zentroparietalen Regionen beobachtet und trat nicht in der Kontrollgruppe auf. Durch eine Quellenlokalisierung konnte zudem eine geringere elektrokortikale Aktivität im posterioren Gyrus cingulare, in der Insula und im Precuneus in der Bettruhegruppe beobachtet werden. In der dritten Studie konnte zudem gezeigt werden, dass die Aufmerksamkeitsleistung der Probanden in kurzen Phasen der Schwerelosigkeit beeinträchtigt war.

Zusätzlich wurde die Aufmerksamkeitsleistung durch den emotionalen Zustand der Teilnehmer unmittelbar vor dem Parabelflug beeinflusst.

Zusammenfassend liefern die Ergebnisse dieser Dissertation weitere Erkenntnisse über die neurofunktionellen und kognitiven Anpassungen des Gehirns, die nicht nur infolge von mit der Raumfahrt verbundenen Stressoren auftreten können, sondern auch in Situationen, die durch soziale Isolation, Reduktion der körperlichen Aktivität oder durch vestibuläre Defizite gekennzeichnet sind.

Résumé

L'espace est l'un des environnements les plus extrêmes pour l'Homme, et pourtant, aujourd'hui, la conquête de l'espace est devenue un enjeu géopolitique et scientifique. Les nations spatiales s'efforcent d'explorer l'espace profond et de coloniser la Lune et Mars. Ces missions exposent les humains à divers facteurs de stress physiologiques et psychologiques qui peuvent également provoquer des processus d'adaptation fonctionnelle et structurelle dans notre cerveau. Au-delà des conditions gravitationnelles altérées et du rayonnement cosmique, l'activité physique restreinte et l'isolement social dans un espace confiné constituent également un risque potentiel. Pour garantir la sécurité mais aussi le succès de ces missions, il est essentiel d'évaluer les influences des séjours de longue durée dans l'espace sur les ressources cognitives et leurs corrélats neuronaux afin de prévoir les risques potentiels pour la santé.

Dans cette thèse, l'impact de trois de ces facteurs de stress sur les fonctions cérébrales a été examiné dans deux modèles d'étude spatiaux (une étude d'alitement et un vol parabolique).

Le *Papier I* a étudié les effets de *l'Inactivité Physique* sur la mémoire épisodique et ses corrélats neuronaux au moyen de l'imagerie par résonance magnétique fonctionnelle. Les facteurs de stress *Isolement*, *Confinement* et la *Privation Sensorielle* et leur impact sur le traitement affectif et l'activité électrocorticale ont fait l'objet du deuxième article. Dans le *Papier III*, l'impact de microgravité sur les processus attentionnels pendant un vol parabolique a été étudié.

Les résultats ont montré que l'immobilisation à long terme de deux mois modifiait l'activité cérébrale pendant l'encodage et la récupération de la mémoire dans l'hippocampe et le gyrus parahippocampique gauches. Cependant, il a été observé qu'un entraînement intensif, réalisée pendant la période d'immobilisation, peut contrer ces changements. Deuxièmement, l'isolement social et le confinement associés à l'alitement ont provoqué une réduction des amplitudes du potentiel évoqué (PE) lors de la visualisation d'images provoquant de vives émotions. Cet émoussement émotionnel a été observé principalement dans les régions centropariétales et ne s'est pas produit dans un groupe témoin. La localisation a confirmé une activité électrocorticale plus faible dans le gyrus cingulaire postérieur, l'insula et le precuneus dans le groupe alité. Troisièmement, la performance attentionnelle était altérée pendant de courtes périodes d'apesanteur. De plus, la performance attentionnelle était fortement influencée par l'état émotionnel des participants juste avant le vol parabolique.

Les résultats démontrent l'impact négatif des facteurs de stress associés aux vols spatiaux sur les adaptations neurocomportementales. Ils vont également au-delà des applications de la médecine spatiale, par exemple dans des situations où l'activité physique ou les contacts sociaux sont réduits.

Introduction

Extreme environments are characterized by harsh life conditions that are considered to be detrimental and even fatal to higher organisms (Gómez, 2011; Thiel, 2011). For humans, a neutral pH-value, moderate temperatures between 20°C and 35°C, and pressures of 1atm are deemed to be optimal (Thiel, 2011). One of the most extreme and hostile environments for humanity is space. Yet, space faring nations and private entities are striving for deep space travel and human settlement on the moon and beyond. These expeditions expose crew members to yet unknown health and performance risks (Stahn & Kühn, 2021a) that must be identified and addressed. While a large body of research has documented the effects of human spaceflight on the cardiovascular and musculoskeletal system (Nicogossian et al., 2016), an increased focus has been recently laid on the central nervous system (Roberts et al., 2020).

With the present dissertation, I intend to identify the cerebral and cognitive adaptations that occur after exposure to environmental and social stressors faced by astronauts during spaceflight. I will first describe the relevant empirical and theoretical background and explain the different cognitive functions in Chapter I. Following on from that, I will present the main research questions in Chapter II and give an overview of the empirical work describing the three different studies that have formed the basis of this dissertation (cf. Chapter III.). Finally, the results and clinical implications of this work along with its limitations and future directions are discussed in Chapter IV.

Chapter I: Theoretical Background

1.1 Space as an Extreme Environment

Space is considered as one of the most hostile and extreme environments (The European Space Agency, 2004). It is characterized by various stressors including but not limited to ionizing radiation, toxic gases, hypercapnia, altered gravity levels, cerebral fluid shifts, temperature extremes, social isolation and confinement, in addition to chronic stress and sleep deprivation (Strangman et al., 2014; Stahn & Kühn, 2021a). The human body has evolved under Earth's gravity to which it is perfectly adapted. Any alteration of gravity provokes adaptational processes of most, if not all physiological systems (Komorowski et al., 2016). Upon entering into the earth orbit, the neurovestibular system is immediately affected by the abrupt loss of gravity (Paloski et al., 1993). The otolith organs in the inner ear rerceive information about horizontal and vertical linear acceleration. However, the constant loss of the linear acceleration subsequently also leads to a loss of the stimulus for vestibular sensation of vertical orientation resulting in motion sickness, an alteration in spatial abilities, and a deterioration in movement control (Paloski et al., 1993; Reschke & Clément, 2018). The lack of the gravitational stimulus also has an impact on the musculoskeletal system generating similar effects on the human body as physical inactivity or immobilization (Pavy-Le Traon et al., 2007). Further stressors arise from the hostile environment. Due to cosmic radiation and temperature extremes, living and working in space is only possible within specialized habitats and with the support of environmental control and life support systems (Komorowski et al., 2016). Living in such a confined environment, isolated from close confidantes may also harbor negative psychological and neurobehavioral effects that are not yet fully understood and that have been declared as an unmitigated risk by the National Aeronautic Space Administration (NASA) (National Aeronautic Space Administration, 2021).

1.2 Research in Space and Spaceflight Analogs

Research on humans in space has proved to be difficult due to financial and logistical challenges as well as a shortage of suitable subjects and their limited availability. To overcome these challenges, terrestrial analog models permitting further investigation on a larger scale have been designed by space agencies (Van Ombergen et al., 2017). Spaceflight analogs include, inter alia, dry and wet immersion, (head-down tilt) bed rest, parabolic flights, and isolation studies in confined controlled or extreme environments (Hargens & Vico, 2016a, 2016b; Pandiarajan & Hargens, 2020; Shelhamer, 2016; Watenpaugh, 2016). Hereinafter, the focus will be on the analog models *Head-down tilt Bed Rest* and *Parabolic Flight*.

1.2.1 Head-Down Tilt Bed Rest

Head-down tilt bed rest (HDBR) has become a widely used model in space medical research that allows to mimic multiple physiological effects that astronauts are likely to encounter during and after a space mission. The commonly used angle of -6° is based on previous research involving Russian cosmonauts using different tilting angles for comfort, acceptability, and magnitude of response (Pavy-Le Traon et al., 2007). The change in posture induces an upward fluid shift and an unloading of the body's weight similar to that observed in space (ibid.). Further adaptational changes in the musculoskeletal system caused by the immobilization may include a reduction in bone density, muscle mass and muscle strength, and insulin resistance (ibid.). Only recently, HDBR has also been shown to elicit neurophysiological changes identical to those seen in astronauts such as the development of *Spaceflight Associated Neuro-ocular Syndrome* (SANS) (Lee et al., 2019) or alterations in brain tissue density (Roberts et al., 2015). Additionally, participants of HDBR studies experience sensory deprivation. They are semi-isolated and confined[1] to a small space for prolonged periods of time allowing for further investigation of these aspects on cognitive function, behavior, and mental well-being (Pavy-Le Traon et al., 1994).

1.2.2 Parabolic Flight

Originally introduced by Fritz and Heinz Haber in 1950, parabolic flights were mainly used for astronaut training and engineering experiments (Haber & Haber, 1950; Karmali & Shelhamer, 2008). These days parabolic flight is the only "Earth-based" analog model where physiological experiments can be conducted on human subjects under different gravity conditions. Although, technically the gravity is still at 1g, a sensation of so-called "weightlessness"[2] emerges through a specific flight trajectory. During this flight maneuver, a flight trajectory in form of an arc is flown which results in a free fall wherein the acceleration of the aircraft cancels the acceleration due to the gravity along the aircraft vertical z-axis. Due to the lack of the aircraft's reaction force on the passengers, a gravity net level of 0g is achieved and all passengers and objects inside the aircraft fall together with an acceleration rate of 9.81 m/s^2 generating the perception of zero-gravity for approximately 20 to 22 seconds (Karmali & Shelhamer, 2008). This maneuver is typically flown 31 times per flight on three consecutive flight days during a parabolic flight campaign. Each microgravity phase is preceded and

[1] Social contacts of probands participating in HDBR studies were restricted to phone/video calls with family/friends, and in-person contacts with medical staff, research teams, and other study participants.

[2] The term "weightlessness" refers to the pure sensation that is induced by the parabolic flight trajectory, and is often used interchangeably with the terms micro-, hypo-, or zero-gravity in Space Life Science. For this reason, this approach will also be followed here, despite these terms technically being incorrect as the gravity level is still 1g.

followed by a hypergravity phase of approximately 1.8g lasting for about 20 seconds each. The characteristic profile of a parabolic flight maneuver is displayed in Figure 1.

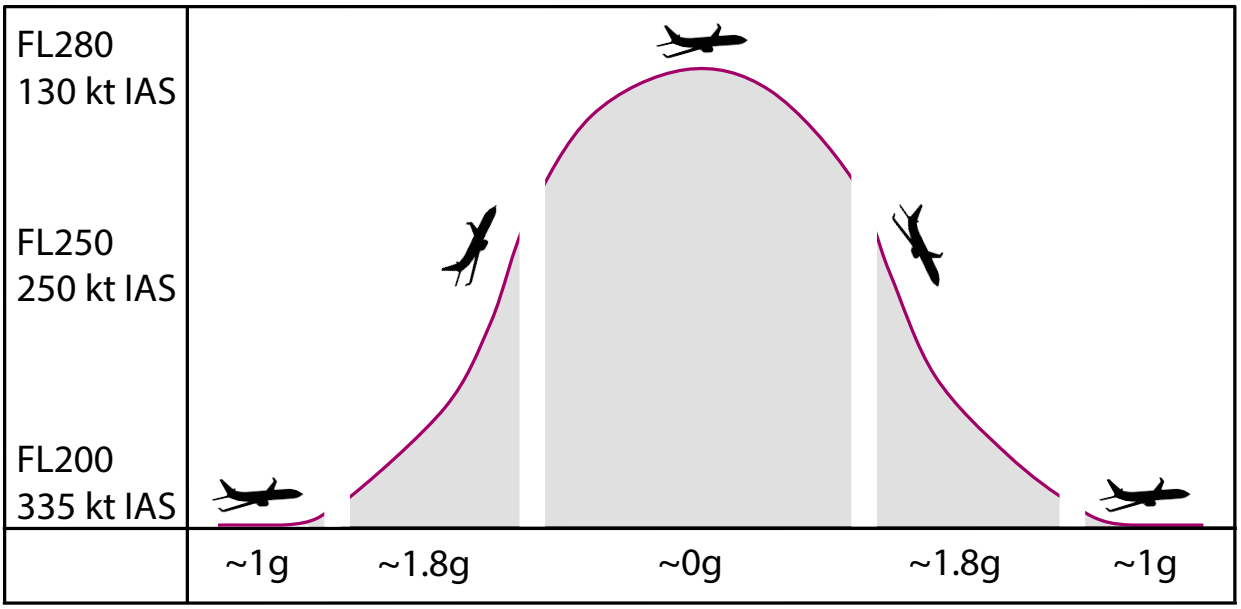

Figure 1: The characteristic profile of a parabolic flight maneuver.
During each parabolic flight maneuver the aircraft starts from a horizontal flight position at a flight level of ~ 200 (approximately 6100 meters). By pulling up the aircraft to an angle of 47° a gravito-inertial acceleration of maximal 1.8g is induced. After 20 seconds the engine's thrust is reduced, and the aircraft enters a free fall trajectory for about 20–22 seconds. During this period the aircraft and all passengers and objects inside of it fall with an acceleration rate of 9.81 m/s², achieving a net 0g-level. This phase is followed by another hypergravity phase (~1.8g) of 20-22 seconds before returning to a steady flight mode of 1g. FL, Flight level; kt IAS, indicated air speed in knots (FL200/ 335 kt IAS corresponds to 825 km/h at 6100 meters above sea level, FL280/ 130 kt IAS corresponds to 370 km/h at 8550 meters above sea level). For further technical details, please see Karmali & Shelhamer (2008) or Pletser (2013).

1.3 Cognition and Neuroplasticity

1.3.1 Cognition

Cognitive functions, conscious and unconscious brain processes involving the domains of perception, memory, learning, attention, decision making, and language abilities (Kiely, 2014) are critical for our daily life and social behavior. They naturally undergo changes throughout the lifespan, i.e., they improve from early childhood to adulthood and may decline after reaching a peak (see Nouchi & Kawashima (2014) for review), but can also be altered by external factors such as someone's lifestyle or various pathologies. Emotion is traditionally not classified as a cognitive function, though it is seen as having a strong impact on cognitive functioning (Tyng et al., 2017). A short overview of *Memory*, *Attention*, *Emotion* and *Affect* will follow hereinafter.

Episodic Memory: As a "true marvel of nature" – this is how Tulving describes episodic memory (Tulving, 2002). It is the only possibility for the human to travel back in time, to re-experience events and situations, and to connect the past with the present (ibid.). Along with semantic memory (the memory of general knowledge and facts), episodic memory (the autobiographical memory of personal events or experience including the information of the *what*, *where*, and *when* (ibid.)), is part of the declarative memory. These two types of memories involve the conscious and intentional recall of previously stored information and are distinct from implicit memory which encompasses the unconscious and effortless recall of information including habits, acquired skills, priming, and conditioning (Bisaz et al., 2014; Milner et al., 1998). The different types of memories are mediated by distinct neural systems throughout the brain (Bisaz et al., 2014). Whereas implicit (non-declarative) memory is associated with neocortical structures, the striatum, or the cerebellum respectively, declarative memory is supported by the medial temporal lobe including the hippocampus (see Figure 2).

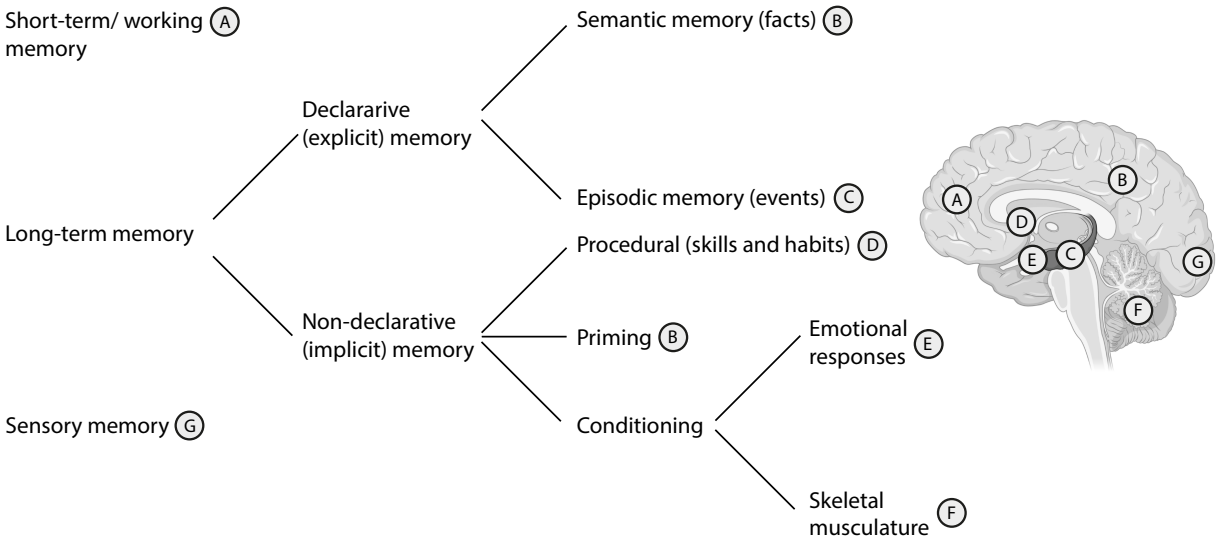

Figure 2: A taxonomy of memory systems listing the brain structures thought to be especially important for each kind of memory system.
A, Prefrontal Cortex; B, Neocortex, C, Hippocampus; D, Striatum; E, Amygdala, F, Cerebellum; G, Primary visual cortex. Please note, that this is a simplified overview, e.g., other regions of the medial temporal lobe play an important role in episodic memory processes; semantic memory is thought to be supported in various cortical and subcortical areas; and sensory memory processes are mediated in distinct brain areas depending on the processing sensory organ. Modified after Milner et al., 1998.

The predominant role of the hippocampus in episodic memory has first been discovered by Scolville & Milner (1957) where lesions in bilateral hippocampi of the prominent patient HM have resulted in the inability for him to form new memories (Scoville & Milner, 1957). Successful encoding and subsequent retrieval of information are facilitated by two mnemonic processes – *pattern separation* and *pattern completion*. *Pattern separation* is defined as the ability to store fast, non-overlapping

representations of similar items or events (Motley & Kirwan, 2012) reducing interference, and thus enabling efficient memory retrieval without retrieving memories with overlapping similarity (McClelland et al., 1995; Motley & Kirwan, 2012). In contrast, *pattern completion* permits retrieving memories from a partial or degraded cue (Motley & Kirwan, 2012; Rolls, 2013). The concept of *pattern separation* and *completion* is displayed in Figure 3. High-resolution neuroimaging studies have shown that both mnemonic processes are best understood in the dentate gyrus and CA3 region of the hippocampus. However, other cortical structures, occipital regions as well as the prefrontal cortex are also known to contribute to successful memory retrieval (Pidgeon & Morcom, 2016). A standardized *pattern separation* and *completion* paradigm was administered to assess mnemonic processes and their neural correlates as part of the study described in *Research Paper I*.

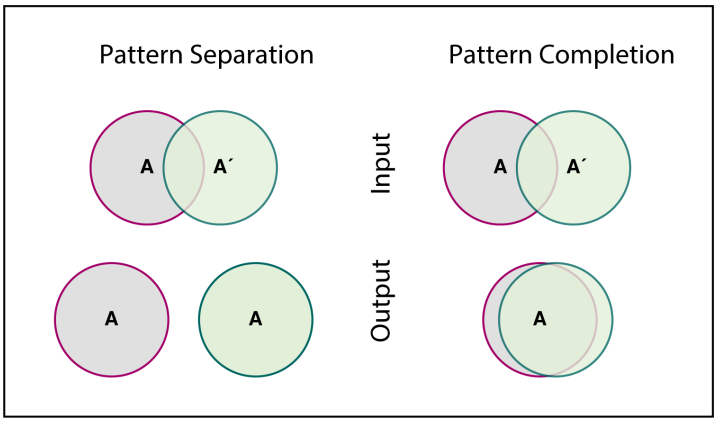

Figure 3: Concept of Pattern Separation and Pattern Completion.
Pattern separation is assumed to be making similar, overlapping items or events more distinct (i.e., A and A'). *Pattern completion* is assumed to be making overlapping representations even more overlapping. Modified after Yassa & Stark (2011).

Attention: Memory and attention are strongly connected: An object or situation must be attended to to be encoded. In turn, memories from past experiences direct which elements should be attended to (Chun et al., 2007). Attention is described as a cognitive process that enables the selection of, the focus on, and sustained processing of information while ignoring other perceivable stimuli (Cohen, 2011). Attention is not a cognitive function *per se*, but is comprised of several processes or subfunctions. These include *Alertness*, *Selective* or *Focused Attention*, *Divided Attention*, and *Sustained Attention* and *Vigilance* (Van Zomeren & Brouwer, 1994). While *Alertness* is considered as the general state of awareness permitting a quick response and reaction to external stimuli, *Selective* or *Focused Attention* describes the ability to focus on certain stimuli (internal or external) ignoring or suppressing irrelevant ones (Bater & Jordan, 2020; Murphy et al., 2016). The ability to simultaneously integrate multiple stimuli is referred to as *Divided Attention* (Iacoboni, 2005). There are various definitions for *Vigilance* with the most common being *Sustained Attention*, i.e., the ability to attend to a stimulus or to

perform a repetitive activity over a longer period of time (Oken et al., 2006). The level of *Alertness*, *Selective Attention*, and *Vigilance* were measured in the study of *Research Paper III*.

Affect, Emotion, and Mood: The terms *Affect*, *Emotion*, and *Mood* are often used interchangeably but there are in fact notable differences. *Affect* is the umbrella term for *Emotions* and *Mood*, and can be distinguished between *Valence* (the extent to which an emotion is pleasant or unpleasant) and *Arousal* (Kleinstäuber, 2013). *Valence* is usually measured by the individual's self-reported data, whereas, *Arousal* can additionally be measured by physiological parameters (e.g. via heart rate) (Niven, 2013). *Emotions* and *Mood* are typically gauged by assessing facial expressions or through eliciting physiological or cognitive responses. They differ in their intensity, duration and eliciting cause (Niven, 2013). Emotions are short but intensive experiences that occur in response to a particular stimulus. Moods, however, are of a longer duration and less intense than emotions.

In the study described in *Research Paper II,* affective processing and its neural underpinnings were measured using stimuli of the *International Affective Picture System (IAPS)*(Lang, Bradley, & Cuthbert, 2008). In the study covered in *Research Paper III*, self-reported moods and anxiety states were recorded using the *Profile of Mood States* (POMS) (McNair et al., 1971) and the *State-Trait Anxiety Inventory* (STAI) (Spielberger et al., 1983), while stress levels were assessed by means of measuring salivary cortisol levels.

1.3.2 Neuroplasticity

Neuroplasticity is the essential characteristic of the brain to adapt and change in response to novel stimuli, and is assumed to be triggered by biochemical and pharmacological factors, alterations in neuronal networks as well as through the generation of new neurons (De Oliveira, 2020; Trojan & Pokorný, 1999). The understanding of neuroplasticity has changed continiously throughout the last centuries. In the late nineteenth and early twentieth century, plasticity was commonly referred to as the acquisition of new skills or the improvement thereof, shaped by personal experiences and circumstances (Merzenich et al., 2013). About half a century later, it was thought that brain changes were limited to a critical period in childhood (ibid.). It took another 50 years until a fundamental rethinking took place brought about by a study of Erickson and colleagues who for the first time could prove the generation of new cells in the dentate gyrus of the hippocampus in the adult human brain (Eriksson et al., 1998). In this study, human brain tissue was obtained postmortem from five cancer patients (mean age: 64.4 ± 2.9 years) who had received a bromodeoxyuridine (BrdU) for diagnostic purposes. Integrated into the DNA of dividing cells, BrdU could be identified by means of immunohistochemistry in the subsequently developed cells thereof (ibid.). Since then, further research

has been conducted to identify factors eliciting neuroplasticity. For instance, van Praag and colleagues have shown that both, physical exercise as well as an enriched environment can induce the genesis of new neurons and their growth (van Praag et al., 2000; Vivar & van Praag, 2017). Chronic stress, sleep deprivation or reduced social interaction in a deprived environment were identified as factors that have adverse effects on the brain (Cinini et al., 2014; Kamal et al., 2014; Maire et al., 2015; Matsumoto et al., 2012; Scaccianoce et al., 2006). These factors have been also identified as possible stressors during spaceflight, but their impact on the brain has only been the center of attention in the past five years (cf. Chapter 1.4). Possible reasons may be the resurgence of public interest in space exploration and the effects thereof on the human body as well as technical advancements of non-invasive measurement methods such as magnetic resonance imaging (MRI), positron emission tomography (PET), or electroencephalography (EEG). These methods have enabled detailed measurements of functional and structural neuroplasticity *in vivo* in humans (Keller & Just, 2016).

1.4 Neurobehavioral and Cerebral Changes in Response to Spaceflight and Spaceflight Analogs

To date, only a few research groups have explored the adaptations of the brain in response to spaceflight or spaceflight analogs (Stahn & Kühn, 2021b). Among them were Koppelmans and colleagues who observed similar changes in gray matter (GM) after 70 days of head-down tilt bed rest and after spaceflight. For both, they reported an increase in gray matter volume of motor and somatosensory areas and widespread decreases around the frontal and temporal pole and the orbits (Koppelmans et al., 2016; Koppelmans, Bloomberg, et al., 2017; Koppelmans, Pasternak, et al., 2017). A reduction in gray matter volume in the orbitofrontal and temporal cortexes was also found in cosmonauts after spaceflight by Van Ombergen et al. along with white matter (WM) decreases of the left temporal pole (Van Ombergen et al., 2018). A retrospective analysis of ISS and space shuttle astronauts revealed white matter changes in the right superior and inferior longitudinal fasciculi, the corticospinal tract, and cerebellar peduncles (Lee, 2019). Contrasting these studies, no changes in total gray or white matter were reported after spaceflight by Roberts et al. (2019). A new approach applied by Jillings and colleagues (2020) could provide an explanation of the divergent results. Using multi-tissue spherical deconvolution, Jillings et al. extracted voxel fractions of the different tissue types within the same voxel instead of assigning one voxel to one tissue type only. The results provide evidence that there is no loss of gray or white matter in the temporal and frontal lobes or around the ventricles, but rather a morphological effect of the fluid redistribution on gray matter (Jillings et al., 2020).

Studies addressing changes in cerebrospinal fluid (CSF) in response to spaceflight or terrestrial analogs are more consistent. Multiple neuroimaging studies have revealed that head-down tilt bed rest as well as spaceflight elicit increased ventricular volumes (Alperin et al., 2017; Hupfeld et al., 2020; Kramer et al., 2020; Riascos et al., 2019; Roberts et al., 2015; Roberts et al., 2019; Van Ombergen et al., 2018; Van Ombergen et al., 2019). These changes were more pronounced with increasing time spent in space (Hupfeld et al., 2020; Roberts et al., 2019) and remained elevated 7 and 12 months after astronauts' return (Kramer et al., 2020; Van Ombergen et al., 2018; Van Ombergen et al., 2019). Roberts and colleagues also noted that astronauts of long-duration missions (time spent in space: 164.8 ± 18.9 days) more likely exhibit an upward shift of the brain and narrowing of the central sulcus and CSF space around the vertex than astronauts of shorter missions (time spent in space: 13.6 ± 1.7 days) (Roberts et al., 2017).

In addition to the studies exploring structural adaptations, some of them have also focused on functional brain changes in response to spaceflight or spaceflight analogs. The first MRI study published, investigating brain changes in response to spaceflight, was that of a 44-year old male cosmonaut. After more than five months (time spent in space: 169 days), Demertzi et al. (2016) observed a reduced *Intrinsic Connectivity Contrast* in the right insula and ventral posterior cingulate cortex as well as a reduced functional connectivity between the left cerebellum and the precentral and the postcentral gyri (Demertzi et al., 2016). Moreover, Demertzi and colleagues (2016) noted an increase of neural activation in the supplementary motor area during a motor imagery task. The results of this single case study confirmed earlier findings from a 45-days bed rest study by Zhou et al. (2014). Pechenkova et al. performed functional magnetic resonance imaging (fMRI) during plantar stimulation before and after long-duration spaceflight (average time spent in space: 183 days ± 55) and observed a decreased functional connectivity of the cerebellum and a decreased connectivity between the vestibular nuclei and sensory and motor regions (Pechenkova et al., 2019). Short bouts of altered gravity levels during parabolic flight induced a lower *Intrinsic Connectivity Contrast* in the right temporo-parietal junction that is involved in multisensory integration and spatial tasks (Van Ombergen et al., 2017). Another study using resting state fMRI during head-down tilt bed rest showed increases in functional connectivity between the right superior parietal gyrus and the left postcentral gyrus (Koppelmans et al., 2018). Task fMRI allows for investigation of the neural underpinnings while participants are engaged in a cognitive task. Overall though the number of studies is rather limited. For example, an increased brain activity in frontal and parietal regions was associated with slower reaction times in a dual task paradigm suggesting a higher neurocognitive demand after 70 days of

head-down tilt bed rest (Yuan et al., 2016). Similarly, a greater activation in the ventromedial prefrontal cortex during risky decision making was observed by Rao et al. (2014).

Studies examining only cognitive function in spaceflight analogs yielded varying results. In 2009, Lipnicki and Gunga reviewed the results of 17 bed rest studies of which six were performed in a head-down tilt position reporting levels of deterioration of (Asiamolov et al., 1986; Ioseliani et al., 1985; Seaton et al., 2009), improvements of (DeRoshia & Greenleaf, 1993; Pavy-Le Traon et al., 1994), or having no effect (Shehab et al., 1998) on cognitive performance (Lipnicki & Gunga, 2009). The majority of the findings were characterized by a strong variability between individuals. In the same vein, Strangmann, Sipes, and Beven summarized studies focusing on cognitive performance in spaceflight and analog environments. Again, these studies did neither show clear evidence nor did they refute the presence of cognitive deficits (Strangman et al., 2014). Though it is consistently suggested that the novel environment induces alterations in cognitive performance, it remains to be fully understood if and to what extent spaceflight is associated with cognitive dysfunction (Strangman et al., 2014).

Chapter II: Summary and Research Questions

Space is one of the most extreme environments for human beings, yet space faring nations continue to strive for deep space exploration and human settlement on Moon and Mars. During space travel astronauts are exposed to various stressors such as altered gravity conditions, radiation, sensory deprivation, and social isolation and confinement. The physiological adaptations of these stressors on the human body has been subject of extensive previous research. Recently, there is an increasing interest in understanding how the brain adapts to this extreme environment. Experiments performed on astronauts and in spaceflight analogs indicate an upward shift of the brain, increased ventricular volumes, morphological redistribution of gray matter and altered intrinsic connectivity in somatosensory and vestibular cortices (cf. Chapter 1.4). Data on cognitive function are more inconsistent ranging from maladaptive effects to no change or even cognitive enhancement and the functional neural substrates of these cognitive changes, if any, have only been minimally explored so far.

For this reason, three different stressors that have been associated with spaceflight were chosen and examined for this dissertation project. Each stressor was addressed in a specific analog model, i.e., head-down tilt bed rest for *Research Paper I* and *II* and parabolic flight for *Research Paper III*. Head-down tilt bed rest is characterized as a *semi-isolated*, *confined* and *sensory deprived environment* with strictly *limited mobility*. Hence, this model was opted for *Study I* and *II* to assess its effects on episodic memory and affective picture processing and their neural correlates, respectively. *Study III* focused on the stressor of *Microgravity* and investigated the effects of altered gravity conditions on attentional processes (cf. Chapter III).

The core aim of this dissertation is to explore how brain function and its neural correlates change in response to spaceflight associated stressors. In particular, the overarching research questions were as follows:

1) How does long-term head-down tilt bed rest affect episodic memory and its neural substrates?

2) What are the effects of bed rest associated sensory deprivation and social isolation and confinement on affective processing?

3) How do short bouts of *Microgravity* impact attentional processes?

The present dissertation explores these questions in three different papers published between 2019 and 2021 (Friedl-Werner et al., 2020; Brauns et al., 2019; and Friedl-Werner et al., 2021).

Chapter III: Overview of Publications

3.1 Research Paper I

Friedl-Werner, A., Brauns, K., Gunga, H.-C., Kühn, S., & Stahn, A. C. (2020). Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest. *NeuroImage*, *223*. doi:10.1016/j.neuroimage.2020.117359

In this research paper, the effects of physical (in)activity on mnemonic processes by means of functional magnetic resonance imaging were investigated. The data was collected as part of the ESA sponsored 60 days bed rest study "Reactive jumps in a sledge jump system as a countermeasure during long-term bed rest" (RSL).

3.1.1 Theoretical background

Over the last two decades a considerable amount of research has shown the beneficial effects of physical exercise on the brain, in particular on the hippocampus (Firth et al., 2018). It has been proven that regular physical exercise can lead to increases in hippocampal gray matter and improve a variety of cognitive functions, including those related to the hippocampus such as learning and memory (Curlik & Shors, 2013; Erickson et al., 2011; Firth et al., 2018). Reversely, a sedentary lifestyle has been associated with adverse effects on hippocampal plasticity, learning, and memory formation as evidenced, inter alia, in cross-sectional studies by Erickson et al. (2009, 2010) (cf. Friedl-Werner et al., 2020). However, these studies did not consider potential confounders. For instance, environmental enrichment (Fabel et al., 2009; van Praag et al., 2000) and social interaction (Djordjevic et al., 2009; Mumtaz et al., 2018) have been reported to further amplify neuroplasticity and episodic memory, whereas, stress and sleep deprivation have been associated with adverse effects (Kamal et al., 2014; Maire et al., 2015; Matsumoto et al., 2012). These factors have not been accounted for during cross-sectional studies and are rather challenging to control for in ambulatory interventional training studies. The terrestrial spaceflight analog model *head-down tilt bed rest* provides a unique possibility to standardize potential confounders such as environmental conditions, wake/sleep cycles, social interaction, and diet.

3.1.2 Hypotheses

The randomized-controlled study investigated the effects of two months of head-down tilt bed rest on episodic memory and its neural correlates. Given that prolonged bed rest may lead to an acceleration of physiological aging processes (Pavy-Le Traon et al., 2007), and that previous studies have reported an age-associated decline in episodic memory (Hämäläinen et al., 2007; Miller et al., 2008; Nyberg et al., 2019; Yassa et al., 2011), an impaired behavioral performance along with functional brain changes in the hippocampus and parahippocampal gyrus were expected. Based on current knowledge about the beneficial effects of physical exercise on the brain, it was additionally hypothesized that a high-intensity exercise training would reduce or even reverse the expected maladaptive effects.

3.1.3 Methods

The study was sponsored by the European Space Agency and conducted at the:envihab facility of the German Aerospace Center (DLR) in Cologne, Germany. The study protocol was approved by the Ethics Committee of the Northern Rhine Medical Association (Ärztekammer Nordrhein) in Düsseldorf, Germany and by the Ethics Committee of the Charité - Universitätsmedizin Berlin, Germany, and has been recorded on the German Clinical Trials Register (DRKS, registration number DRKS00012946, 18th of September 2017) (cf. Friedl-Werner et al., 2020).

Twenty-two men were exposed to 60 days of uninterrupted head-down tilt bed rest. Half of the participants were additionally assigned to a high-intensity interval training that was performed five to six times per week throughout the bed rest period (TRAIN: n = 11, age: 30.8 ± 6.2 yrs), while for the other half there was no intervention during the bed rest phase (CTRL: n = 11, age: 28.2 ± 5.7 yrs) (cf. Friedl-Werner et al., 2020). Neuroimaging was performed four days prior to and 58 days after bed rest using a 3 Tesla Siemens Biograph mMR. Blood oxygenation level dependent (BOLD) changes in the brain tissue were measured using an echo planar imaging sequence (EPI) while the participants performed two blocks of a pattern separation paradigm which was first introduced by Kirwan and Stark in 2007 (Kirwan & Stark, 2007). During this task, pictures of different objects were presented to the participants. Participants had to decide for each picture, whether they had seen the object *before*, *not at all*, or a *similar one*, by pressing a button. This pattern separation paradigm has been reported to reliably target the hippocampus in previous studies (Ally et al., 2013; Bakker et al., 2008; Kirwan et al., 2012).

Data was preprocessed and analyzed using statistical parametric mapping (SPM12). Two different general linear models were generated within the SPM framework. The first model included

a *Whole Brain Analysis* based on the *pattern separation* and *completion* contrasts proposed by Pidgeon and Morcom (Pidgeon & Morcom, 2016). The second model was based on the different stimuli conditions as specified by Kirwan and Stark (2007). The model pursues a hypothesis-driven approach defining the bilateral hippocampus and parahippocampal gyrus *à priori* as *Regions of Interest* (ROI). For each ROI, each participant, condition, and point in time, the mean BOLD signal change covering a time window of 4 to 6 seconds after stimulus onset was extracted using the marsbar toolbox (Brett et al., 2002). Extracted values were further used in a linear mixed model using *Time* (BDC-4, HDT58), *Group* (TRAIN, CTRL), *Laterality* (Left, Right), *Condition* (Encoding, Retrieval), *Stimulus Type* (Lure, Repetition) as fixed factors, and *Subject* as a random factor (cf. Friedl-Werner et al., 2020). Detailed magnetic resonance (MR) specification, preprocessing as well as analyzing steps are outlined in the article Friedl-Werner et al. (2020).

3.1.4 Major findings

In line with the hypothesis, changes of the BOLD signal were observed. CTRL showed an increase of brain activation, while the signal in TRAIN decreased during the encoding and retrieval process. A significant *Time* x *Group* interaction effect was found in the left hippocampus and parahippocampal gyrus. Contrary to the second hypothesis, no differences in the mnemonic performance between the two groups could be observed.

3.2 Research Paper II

Brauns, K., **Werner, A.**, Gunga, H.-C., Maggioni, M. A., Dinges, D. F., & Stahn, A. (2019). Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization. *Scientific Reports*, *9*(1). doi:10.1038/s41598-019-52555-1

In this research paper, it was sought to explore the effects of one month of head-down tilt bed rest on affective picture processing by means of electroencephalography. The data was collected as part of the ESA sponsored 60 days bed rest study "Effects of a nutritional cocktail consisting of antioxidant and anti-inflammatory supplements to prevent the deconditioning induced by 60 days of antiorthostatic bed rest (Cocktail)". A cross-sectional design that was unrelated to the antioxidant countermeasure of the overall research protocol was employed.

3.2.1 Theoretical background

During long-duration spaceflight, astronauts encounter various stressors such as reduced sensory stimulation, social isolation and confinement. Sensory deprivation, monotony and boredom in isolated and/or confined settings are thought to be major contributors to adverse neurobehavioral conditions that can lead to interpersonal tension and conflict, negative affect, work place errors, and even to an increased mortality (Eastwood et al., 2012; Stahn & Kühn, 2021a). Supporting this, previous research in spaceflight analogs has shown increased levels of depression, altered mood states (Ishizaki et al., 2002), and impaired emotion recognition (Basner et al., 2021; Liu et al., 2012). Affective processing, emotion recognition and regulation are essential to human behavior as they enable us to communicate and interact with fellow humans. Any dysfunction or alteration of emotional processing may not only hamper astronauts' mental well-being, but also affect their cognitive performance which in turn can jeopardize the success and safety of spaceflight missions as a whole (cf. Brauns et al., 2019).

3.2.2 Hypotheses

The terrestrial spaceflight analog HDBR encompasses monotony, sensory deprivation, semi-isolation, and confinement. Studies conducted during long-term bed rest indicate the risk of developing mood disorders (Ishizaki et al., 2002) and a deterioration of emotion recognition (Liu et al., 2012). It was therefore hypothesized that one month of head-down tilt bed rest would lead to a cortical inhibition of affective picture processing, indicated by reduced event-related potentials (ERP) as well as changes in self-reported evaluations of the affective stimuli.

3.2.3 Methods

The study was sponsored by ESA and carried out at the French Institute for Space Medicine and Physiology (MEDES), Toulouse, France. The overall project was registered as a clinical trial in the clinical trial database (www.clinicaltrial.gov; identifier: NCT03594799), and was approved by the Comité de Protection des Personnes (CPP Sud-Ouest Outre-Mer I), the French Health Authorities (Agence Française de Sécurité Sanitaire des Produits de Santé), and the Ethics Committee at Charité – Universitätsmedizin Berlin (cf. Brauns et al., 2019).

Twenty healthy men were included in the study and were tested either before (CTRL: n = 10; 34 ± 7 yrs) or after 31 days of head-down tilt bed rest (HDBR: n = 10; 34 ± 8 yrs). A total of 75 colored photographs (25 pleasant (e.g., sporting events, erotic scenes), unpleasant (e.g., scenes of violence, threat and injuries), and neutral (e.g., household objects)) selected from the standardized *International Affective Picture System* (IAPS) (Lang, Bradley, & Cuthbert, 2008) were presented in a random order to the participants while electrocortical activity was continuously recorded (cf. Brauns et al., 2019). For each photograph, participants were asked to rate the *Arousal* and *Valance* on a 9-point *Self-Assessment Manikin* rating scale. Electrodes were attached to an EEG cap (actiCap, Brain Products GmbH, Germany) and electrocortical activity was recorded and synchronized with the stimuli using an active electrode 32-channel amplifier (actiCHamp, Brain Products GmbH, Germany) (cf. Brauns et al., 2019). Data was preprocessed and artefacts were removed using EEGLAB version 14.0.0. For each subject and each condition, the average ERP (P300 and late positive potential (LPP)) were computed separately. Waveforms were transformed into topographic maps of the ERP potential distributions (cf. Brauns et al., 2019).

Behavioral data for emotional *Valence* and *Arousal* as well as ERP components (P300 and LPP) were each entered separately into a linear mixed model using *Subject* as a random factor, and *Group* (CTRL, HDBR) and *Stimulus Type* (pleasant, unpleasant, neutral) as fixed factors. To identify the spatial localization of electrocortical activity, a source analysis using the eLORETA software was performed. Independent sample *F*-tests were used to test for differences in estimated *Cortical Current Density* between groups for each emotional classification (pleasant, unpleasant, neutral), and for LPP and P300, respectively. Detailed preprocessing as well as analyzing steps are described in the article Brauns et al. (2019).

3.2.4 Major findings

As expected, emotional stimuli induced larger P300 and LPP amplitudes as neutral ones in the control group. In contrast, participants tested after one month of bed rest showed an emotional blunting, i.e., pleasant and unpleasant stimuli elicited similar electrocortical responses as neutral pictures. This emotional blunting was observed predominantly in centroparietal regions. Additionally, a reduced lower activity in the bilateral posterior cingulate gyrus, insula and precuneus was observed in the HDBR group compared to the control group for both ERP components for the pleasant and unpleasant, but not for the neutral stimuli.

3.3 Research Paper III

Friedl-Werner, A., Machado, M.-L., Balestra, C., Liegard, Y., Philoxene, B., Brauns, K., Stahn, A. C., Hitier, M., & Besnard, S. (2021). Impaired Attentional Processing During Parabolic Flight. *Frontiers in Physiology*, *12*, 675426. doi:10.3389/fphys.2021.675426

In this research paper, the impact of short bouts of acute weightlessness on attentional performance was investigated while seeking to identify potential confounders linked to the parabolic flight experiment. The data was collected during the 131st parabolic flight campaign.

3.3.1 Theoretical background

The impact of altered gravity levels during parabolic flight on cognitive functions has been subject to previous research yielding divergent results. For instance, improvements of reaction times during a complex mental arithmetic task in combination with an Oddball paradigm have been reported by Wollseiffen and colleagues (Wollseiffen et al., 2016, 2019). Clément et al. (2016), Grabherr et al. (2007), Grabherr & Mast (2010), and Stahn et al. (2020), in contrast, found that spatial cognitive abilities seem to have been negatively affected by altered gravity conditions during parabolic flight. Attentional processes are thought to influence executive functions, working-memory and spatial cognition. Thus, changes in attentional capacity could therefore directly affect other cognitive domains. The impact of parabolic flight on attentional processes has to date not been subject to detailed research. Besides altered gravity conditions, the experimental setting of a parabolic flight itself is unique and produces various psychological stressors which may result in participants experiencing severe motion sickness, an increase in perceived stress, mood changes, and a deterioration of sleep quality. These factors can additionally affect attentional performances. Accordingly, it is crucial to unravel the effects related to microgravity from potential confounders associated with parabolic flight maneuvers in order to better understand the impact of altered gravity conditions on neurobehavioral performance (cf. Friedl-Werner et al., 2021).

3.3.2 Hypotheses

The study investigated attentional processes during short bouts of weightlessness during parabolic flight. It was hypothesized that performance of attentional processes would be impaired by the state of weightlessness. Owing to the experimental set-up of a parabolic flight campaign, associated

elements such as sleep quality, mood and anxiety states, stress levels as well as the use of scopolamine were additionally assessed and were expected to affect the level of alertness and attentional processes.

3.3.3 Methods

Data was collected during the 131st parabolic flight campaign which was sponsored by the French space agency CNES (Centre national d'étude spatiales) and operated by Novespace. The campaign took place in Bordeaux Merignac over three consecutive flight days (cf. Friedl-Werner et al., 2021). The theoretical background of a parabolic flight has been described in Chapter 1 (cf. 1.2.2). Included in the analysis was data of twelve men (mean age: 48.75 yrs ± 8.7) who were novices to the parabolic flight experience. To measure attentional performances a *Continuous Performance Task* (CPT) was completed at different points in time, prior to, during and after exposure to the parabolic flight. Attentional performance was quantified using the parameters of (1) *Reaction Time* (RT) for target trials, (2) *Hit Rate* (correct detection of the target letter) to determine attentional capacity, (3) *False Alarm Rate* (Reaction to non-targets), and (4) *d-prime* (d') as an indicator of sensitivity (cf. Friedl-Werner et al., 2021).

Motion sickness susceptibility, anxiety levels, mood states, and sleep quality were assessed using self-reported standardized questionnaires. Participants' stress responses were quantified through measuring salivary cortisol concentrations. To investigate whether the use of scopolamine, which is administered as a voluntary routine option to prevent motion sickness, impacts attentional performance and vigilance, the CPT and a psychomotor vigilance test (PVT) were employed before and after the subcutaneous injection of 0.175 mg scopolamine. A linear mixed model with *Time* as a fixed and *Subject* as a random factor was used to assess differences between points in time, followed by the calculation of pre-planned contrasts with a sequential Holm – Bonferroni correction for multiple comparisons (Holm, 1979). A repeated measures correlation was conducted to verify whether an association can be identified between CPT variables and mood and anxiety states, and CPT variables and stress levels (cf. Friedl-Werner et al., 2021).

3.3.4 Major findings

The findings of the study suggest that short bouts of weightlessness hamper attentional processes as evidenced by reduced *Reaction Times* and *Hit Rates*, and higher *False Alarm Rates* of the CPT. Participants' arousal and anxiety states significantly increased prior to the flight and decreased immediately after parabolic flight exposure. Additionally, higher levels of anxiety were moderately

associated with a higher deterioration of CPT performance parameters. The use of scopolamine only minimally affected participants' vigilance, but not their actual attentional capacity.

Chapter IV: Discussion

This chapter will see a discussion of the key findings as well as the endeavor to integrate those findings within the existing body of literature. I will also address possible methodological limitations of the studies and touch upon clinical implications which may contribute to the wider medical knowledge. Concluding, I will suggest aspects not covered here, which may be beneficial for the field more broadly and worth exploring further going forward.

4.1 Summary and Evaluation

4.1.1 Physical (in)activity alters brain activity in the left hippocampus and parahippocampal gyrus during memory encoding and retrieval

Over the past two decades, an increasing body of literature has been reporting beneficial effects of physical exercise on the brain. In particular, the hippocampal formation and cognitive functions such as episodic memory or spatial navigation seem to benefit from regular physical exercise (Erickson et al., 2014; Firth et al., 2018). Reversely, in cross-sectional studies children as well as elderly probands following a sedentary lifestyle were shown to have less hippocampal gray matter and perform less well in relational or spatial memory tasks (Bugg & Head, 2011; Chaddock et al., 2011; Erickson et al., 2010; Erickson et al., 2009). *Research Paper I* underpins these findings and provides further evidence of the modulating effect of regular physical exercise on hippocampal and parahippocampal brain activity. While neuronal activity decreased in the bed rest group that was additionally assigned to the exercise intervention, brain activity increased in the bed rest control group not performing this exercise after 58 days of bed rest. A similar pattern was observed in cross-sectional studies comparing patients with mild cognitive impairment (MCI) versus healthy controls (Hämäläinen et al., 2007; Yassa et al., 2010) as well as comparing elderly with young study participants (Yassa et al., 2011). In these studies, Yassa and colleagues applied the same paradigm and observed an increased brain activity in MCI patients and the elderly (Yassa et al., 2010, 2011). The physiological responses to spaceflight and to HDBR are often likened to an accelerated aging process (McGuire et al., 2001; Vernikos & Schneider, 2010). Thus, increases in brain activity may be interpreted as a consequence of bed rest-induced accelerated aging which could be counteracted by high-intensity training (cf. Friedl-Werner et al., 2020). Further evidence for this assumption comes from Miller and colleagues who reported a greater hippocampal activity during memory encoding, predicting the rate of cognitive decline (Miller et al., 2008a). They also associated low encoding performance with greater brain activity in the hippocampus and assumed

that this was a compensatory response (Miller et al., 2008). Other research groups reported different results, i.e., hypoactivity in all hippocampal subfields in Alzheimer patients performing face memory task (Small et al., 1999). Studies carried out by Sperling et al. and Nyberg et al. may offer some explanation of these contrasting results. Sperling suggested that hippocampal hyperactivity during mnemonic processes occurs in the early stages in patients with mild cognitive impairment whereas hypoactivity occurs when the hippocampus fails to maintain the mnemonic performance (Sperling, 2007). Nyberg is of the assumption that hyperactivity in the hippocampus during encoding is not an actual sign of normal aging but rather a pathological sign of underlying neurocognitive disorders (Nyberg et al., 2019). Immobilization and physical inactivity cause similar physiological adaptions in the human body as the absence of the gravitational stimulus in space. The results may, therefore, suggest a potential additional risk on a cerebral level for astronauts on long-duration spaceflights. However, it cannot be conclusively determined to what extent the observed neuronal changes may also affect related cognitive functions and operational activities.

4.1.2 Prolonged bed rest impairs affective picture processing in the right insula, bilateral precuneus, and bilateral posterior cingulate gyrus

The terrestrial spaceflight analog HDBR does not only allow for the investigation of physiological adaptations, but also for psychological ones as it involves the components of monotony, sensory deprivation, semi-isolation and confinement. Studies conducted during long-term bed rest indicate the risk of developing mood disorders (Ishizaki et al., 2002) and a deterioration of emotion recognition (Liu et al., 2012). *Research Paper II* provides greater granularity on impaired affective processes after extended periods of time of bed rest. In the experimental group that underwent 31 days of HDBR, electrocortical activity was inhibited during the processing of emotional (pleasant and unpleasant) stimuli compared to neutral stimuli as evidenced by reduced P300 and LPP components. This cortical inhibition was found in the right insula, bilateral precuneus, and bilateral posterior cingulate gyrus (PCG). In contrast, an age-matched control group that was not exposed to bed rest showed the expected electrocortical response to emotional stimuli, i.e., higher P300 and LPP components compared to a level response when shown neutral photographs (Cuthbert et al., 2000; Schupp et al., 2004). There are several possible explanations for the emotional blunting observed in the HDBR group which may indicate dysfunctional modulations in the processing of emotional information (cf. Brauns et al., 2019). Highly emotional stimuli rapidly capture ones' attention (Carretié et al., 2004), and are therefore more likely to be encoded, consolidated and retrieved (Mackay et al., 2004). Long-term bed rest and physical inactivity have also been associated with impaired attentional processes

(Brauns et al., 2021). It can hence be assumed that the automatic attention, usually responding to emotional stimuli by default, is disrupted by a general deterioration of attentional processes induced by prolonged bed rest. Another explanatory approach is based on the neuroendocrine system. It is suggested that physical inactivity leads to decreases of the neurotransmitters serotonin and norepinephrine (Corcoran, 1991). Serotonin and norepinephrine are known to play a critical role in affecting human behavior (Sedvall, 1980) and in psychiatric disorders such as depression (Sharp, 2008), anxiety (Nutt, 1990), and behavioral deviations among people with dementia (Lai, 2003). Thus, a change in monoamine concentrations associated with long-duration immobilization could also contribute to the observed alterations in visual affective processing (cf. Brauns et al., 2019). Decreases in electrocortical activity were localized in the right insula, bilateral PCG, and bilateral precuneus, regions that are involved in various emotional processes. For instance, the PCG has been found to be activated during participants' evaluation of emotional terms (Maddock et al., 2003), while the precuneus has been shown to be involved in emotional episodic memory (Andreasen et al., 1995) and in self-attribution (Cabanis, 2013). The insula has been associated with the detection, interpretation, and regulation of internal bodily states (Critchley, 2005), and all three regions are reciprocally connected to further areas that are known to be involved in emotional processing such as the anterior cingulate gyrus, the orbital frontal cortices and the amygdala (Baleydier & Mauguiere, 1980; Mufson et al., 1981). Studies attempting to attribute different emotions to different brain areas have demonstrated that the right hemisphere appears to be more responsive to emotional stimuli than the left one, and is more prone to negative emotions such as fear and disgust (Kallat, 2008). The emotional cortical inhibition that was described in *Research Paper II* may have an impact on social interaction and team dynamics in the long run, and thus, may be a potential risk factor for long-duration spaceflight.

4.1.3 Attentional processes are disrupted during weightlessness and are determined by participants' emotional states

Parabolic flight offers the unique opportunity to perform neurobehavioral experiments in human beings under different gravity conditions. Previous research has shown divergent results for different cognitive functions during short bouts of weightlessness. Unfortunately to date, no study has examined attentional processes during parabolic flight, although cognitive and motor performance clearly depend on the level of attention and selective attention abilities (Carrasco, 2018; Ruff & Cohen, 2019; Song, 2019). Additionally, the setting of a parabolic flight campaign involves various additional stressors that are often not considered adequately even though they may affect attentional processes, and in turn, could have an impact on other cognitive domains. *Research Paper III* builds on this aspect

with the research team determining the effect of microgravity on attentional processes, and also evaluating the impact of additional factors associated with the experimental setup of a parabolic flight on same. The performance decline in the *Continuous Performance Task* (CPT) observed before the first parabola and during microgravity may be attributed to participants' highly emotional states as well as to their disrupted vestibular and sensorymotor systems during weightlessness. Inputs from the vestibular, somatosensory, proprioceptive, and visual systems are important for ones' bodily awareness, movement control as well as for spatial orientation (Reschke & Clément, 2018). In microgravity, however, the otolithic response of the vestibular organs is inhibited and proprioceptive information from the musculoskeletal system is lacking (Probst et al., 1996; Reschke et al., 2018), resulting in an erroneous cortical calibration of visual and somesthetic information related to spatial orientation. It can be assumed that the sensory awareness of ones' own body, the control of movements, and the highly emotional state require a higher level of divided attention (cf. Friedl-Werner et al., 2021). There is some evidence to suggest that vestibular deficiency in form of bilateral vestibular deafferentation causes impairments in attentional ability (Zheng et al., 2009). There is also evidence that supports the role of vestibular inputs for emotional processing (Barona-de-Guzmán et al., 2018; Lopez & Blanke, 2011; Rajagopalan et al., 2017) including the fear of falling (Schlick et al., 2016) and panic disorders (Perna et al., 2001). A higher self-reported anxiety state was moderately associated with a declined performance in CPT. Selective and sustained attention did not appear to be significantly impacted by stress levels, sleep quality, and the administration of scopolamine in the setting of a parabolic flight. The results do not allow to draw final conclusions for long-duration spaceflight missions as it is unclear whether prolonged exposure to microgravity would accelerate the decline in attentional processes. Yet, the results provide additional clues that should be considered in behavioral research designs for parabolic flight experiments.

4.2 Limitations

4.2.1 Study design

A tailored experimental design was chosen for each study in order to best address the research questions. Yet, the choice of each respective study design may be subject to discussion. The mixed-design of *Study I* (cf. *Research Paper I*) combined the features of a *within* and *between-subject* design, i.e., to assess the effect of *Time* for each group and to evaluate the efficiency of the exercise intervention. It could be argued that the study would have benefited from an additional control group not exposed to two months of bed rest. This would have allowed us to examine the effects of the long-term immobilization on mnemonic processes. Given that each participant performed the task twice, the

improvement in *item discrimination* seen in both groups may be viewed as a test-enhanced learning effect. It is unclear whether the repeated assessment of the task also influenced the neural signal in some way.

Repeated testing was intentionally waived in *Study II* (cf. *Research Paper II*) to preserve the surprise effect of unexpected nature of the emotional pictures presented to the participants. Yet, any bias due to group heterogeneity cannot be ruled out in this *cross-sectional* design.

In *Study III* (cf. *Research Paper III*), a *within-subject* design was chosen whereby participants served as their own controls. To have a solid baseline, an extensive familiarization session had been carried out with all participants thoroughly explaining the attentional tasks, and no practice effects had been observed. Still, it would have been worthwhile to assess participants' emotional states one week prior to the parabolic flight to have a more reliable baseline. Additionally, the study would have benefited from a comparison with a group of more experienced flyers. In such a cohort, the effect of microgravity may have been more pronounced, and participants' stress and anxiety levels may have made less of an impact on attentional processes.

4.2.2 Sample size and selection bias

The small sample sizes (*Research Paper I*: n = 22; *Research Paper II*: n = 20; *Research Paper III*: n = 12) of the here discussed studies may be seen as a limitation. In spite of that, small samples are common in research in extreme environments, and in particular in space research. Terrestrial spaceflight analogs can provide a practical solution, and although the number of participants involved in these studies is still small, it is in line with previous studies using spaceflight analogs (see Lipnicki and Gunga (2008) for review).

Limited generalizability and selection bias are other important aspects to consider. The three here described studies included only male participants. The overall study protocol of the studies detailed in *Research Paper I* and *II*, including inclusion and exclusion criteria were set by the European Space Agency, the study sponsor. In fact, the majority of HDBR studies have been conducted with men. The main reason for women still being underrepresented in these kinds of studies is women's fluctuating hormonal levels. Due to a drop in estrogen levels and resulting bone density loss during menopause, women have a naturally higher risk of developing osteoporosis which is further exacerbated by physical inactivity such as long-term bed rest. These hormonal fluctuations also make standardized studies more challenging. This has become a "hot topic" in general clinical research. Given that current and future space missions also involve female astronauts, NASA and ESA have recently started to conduct HDBR studies with women albeit the studies being of a shorter duration.

4.2.3 Methodological aspects

Several different methodological approaches were taken, inter alia, by applying fMRI, EEG, and through utilizing questionnaires. Self-reported data obtained through questionnaires and the *Self-Assessment Manikin Rating Scale* are subject to a response bias with probands showing behaviors such as social desirability and acquiescence. Qualitative data often underlies cognitive-social control and may, therefore, not adequately reflect participants' true affective states (Kreitchmann et al., 2019). Functional MRI and EEG offer more objectivity in this respect. That said, the BOLD signal is still an indirect measure of neural activity, and therefore, susceptible to non-neural changes in the body. Functional MRI has a very high spatial resolution though the temporal resolution is rather poor. In event-related designs, blood oxygenation peaks at approximately five seconds post-stimulus. This is slower than the underlying neural processes. Through this delay the temporal information is heavily blurred and can be influenced by other events occurring within the same time window (Glover, 2011). To decrease the physiological noise and movement artefacts, a common pre-processing pipeline was applied in *Study I* (cf. *Research Paper I*). Additionally, the mean percent signal change occurring 4 to 6 seconds after stimulus presentation was extracted to obtain a more robust event-related BOLD response. In contrast to fMRI, EEG has a better temporal resolution and can capture the dynamics of evoked responses immediately, i.e., within milliseconds (Glover, 2011). However, EEG is biased towards the cortical surface and does not allow for a precise localization of brain areas. Recent technical developments of MR compatible EEG hardware now allow for simultaneous EEG and fMRI acquisition which would overcome these challenges and provide precise temporal and spatial information on stimuli processing and the underlying neural correlates (Mele et al., 2019).

4.3 Clinical Implications and Future Directions

The results of the here presented studies may also be relevant for vulnerable populations in a non-space context, and raise important questions for people following sedentary lifestyles or for situations in which physical activity levels are restricted. For instance, in clinical settings, bed rest is still prescribed as a treatment for various patient groups and used as an indispensable instrument in intensive care. Hospitalized older persons can spend as much as 80% of their time confined to bed (Goswami, 2018). Although, elderly and immobilized patients differ from healthy participants partaking in the here presented bed rest studies, the results suggest that prolonged immobilization periods have an adverse effect on episodic memory and affective processing as well as the underlying neuronal signals thereof. Future research should focus on the development of suitable countermeasures. As presented in this dissertation, physical exercise can serve as one such potential

countermeasure, but the intensity and exercise mode should be adapted to the respective patient group. The study outcomes have also shown how fast emotional blunting can occur at the cortical level. Frequent social interaction with family, friends or caregivers as well as the exposure to a stimulating environment may defer or even prevent this negative adaptational process. There is also a growing body of research using virtual reality (VR) in clinical settings which could be an option to mitigate monotony and the experienced sensory deprivation. Without changing location, patients would be given the opportunity to experience a sensory stimulating environment. It is expected that with technological advances, VR applications will see even broader employment in the future.

Research on astronauts to date has already contributed significantly to the understanding of the vestibular system (Miller et al., 1969; Shelhamer, 2016). Astronauts and persons with vestibular deficits face analogous challenges caused by adaptive exogenous (weightlessness-induced), or endogenous (pathology-induced) changes in the processing of acceleration stimuli (Lawson et al., 2016). Vestibular dysfunction is characterized as a multisensory syndrome with static and dynamic postural-motor and psychocognitive symptoms related to the projection of vestibular information at all cerebral levels. Persons with vestibular pathologies do often report limitations in their everyday life consisting of psychological, cognitive and social challenges. Besides a greater functional disability such as orientation and memory loss, attention deficits and concentration problems have been reported by persons suffering from these conditions, which is in line with the outcomes of this dissertation. Given these neurovestibular similarities, both, astronauts and patients with vestibular dysfunctions may therefore benefit from shared research approaches and treatment strategies.

4.4 Conclusion

Three different stressors encountered by astronauts during long-duration missions as well as the stressors' (resulting) impact on the human brain and cognitive functions were examined as part of this dissertation. It was found that prolonged bed rest altered brain activity during memory encoding and retrieval, and lead to impaired affective processing. It was also shown that weightlessness and highly emotional states significantly interfered with attentional performance. The results go beyond applications of space medicine, and may foster an increased understanding for patients with vestibular dysfunctions and of the cognitive and emotional adaptations during hospitalization, as well as aging processes and sedentary lifestyles more generally.

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Statutory Declaration

I, Anika Friedl-Werner, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "Cortical Neurosplasticity and Cognition in Extreme Environments" (Kortikale Neuroplatizität und Kognition in Extremen Umwelten), independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I also declare that this is an International Joint Dissertation *(Cotutelle du thèse)*. Therefore, this dissertation has been also submitted, in identical form to *Université de Caen Normandie.*

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Signature of doctoral candidate

Declaration of Contribution

I, Anika Friedl-Werner, contributed the following to the below listed publications:

Publication I: **Friedl-Werner, A.**, Brauns, K., Gunga, H.-C., Kühn, S., & Stahn, A. C. (2020). Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest. *NeuroImage*, *223*. doi:10.1016/j.neuroimage.2020.117359

Contribution in detail: I preprocessed all neuroimaging and behavioral data using a Matlab and R code. Furthermore, I performed all statistical analyses, interpreted the data, and created the figures and tables shown in *Publication I* including the ones in the supplementary material. I wrote the original draft of the manuscript and edited it accordingly after receiving feedback from all co-authors. I prepared all answers to the reviewers' comments and edited the manuscript before resubmission.

Publication II: Brauns, K., **Werner, A.**, Gunga, H.-C., Maggioni, M. A., Dinges, D. F., & Stahn, A. (2019). Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization. *Scientific Reports*, *9*(1). doi:10.1038/s41598-019-52555-1

Contribution in detail: As a study coordinator, I was involved in preparing the study, i.e., writing the ethical documents, designing the experimental schedule, and requesting quotes for purchasing the technical equipment. In addition, I selected the test stimuli, ran pilot tests before the study start, implemented the experimental protocol, performed all data collection in Toulouse, France, and ensured data quality. I wrote parts of the method sections and edited the manuscript before (re-) submission.

Publication III: Friedl-Werner, A., Machado, M.-L., Balestra, C., Liegard, Y., Philoxene, B., Brauns, K., Stahn, A. C., Hitier, M., & Besnard, S. (2021). Impaired Attentional Processing During Parabolic Flight. *Frontiers in Physiology*, *12*, 675426. doi:10.3389/fphys.2021.675426

Contribution in detail: I preprocessed and analyzed the behavioral data using a self-written R code. In addition, I performed all statistical analyses, interpreted the data, and created all the figures and tables shown in *Publication III* including the ones in the supplementary material. Together with the last author S. Besnard, I wrote the original draft of the manuscript in equal shares. I have integrated the feedback of my co-authors, submitted the manuscript to the journal, and corresponded with the editor and reviewers. I answered all reviewers' comments and edited the manuscript accordingly including further changes from the co-authors.

Signature of doctoral candidate:

Research Paper I

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI
Selected Categories: **"NEUROIMAGING"** Selected Category Scheme: WoS
Gesamtanzahl: 14 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NEUROIMAGE	99,720	5.812	0.132720
2	HUMAN BRAIN MAPPING	22,040	4.554	0.043230
3	NeuroImage-Clinical	5,762	3.943	0.022670
4	Journal of NeuroInterventional Surgery	4,407	3.925	0.011860
5	Brain Imaging and Behavior	2,464	3.418	0.006610
6	AMERICAN JOURNAL OF NEURORADIOLOGY	23,231	3.256	0.028010
7	NEURORADIOLOGY	5,656	2.504	0.007020
8	JOURNAL OF NEURORADIOLOGY	985	2.467	0.001440
9	PSYCHIATRY RESEARCH-NEUROIMAGING	5,503	2.270	0.008330
10	JOURNAL OF NEUROIMAGING	2,081	2.080	0.004270
11	NEUROIMAGING CLINICS OF NORTH AMERICA	1,173	2.046	0.001310
12	STEREOTACTIC AND FUNCTIONAL NEUROSURGERY	1,807	1.905	0.002220
13	CLINICAL EEG AND NEUROSCIENCE	1,018	1.822	0.001510
14	KLINISCHE NEUROPHYSIOLOGIE	40	0.325	0.000030

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Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest

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ABSTRACT

Episodic memory depends decisively on the hippocampus and the parahippocampal gyrus, brain structures that are also prone to exercise-induced neuroplasticity and cognitive improvement. We conducted a randomized controlled trial to investigate the effects of a high-intensity exercise program in twenty-two men resting in bed for 60 days on episodic memory and its neuronal basis. All participants were exposed to 60 days of uninterrupted bed rest. Eleven participants were additionally assigned to a high-intensity interval training that was performed five to six times weekly for 60 days. Episodic memory and its neural basis were determined four days prior to and on the 58th day of bed rest using functional magnetic resonance imaging (fMRI). We found increased BOLD signal in the left hippocampus and parahippocampal gyrus in the non-exercising group compared to the exercising bed rest group whereas the mnemonic performance did not differ significantly. These findings indicate a higher neuronal efficiency in the training group during memory encoding and retrieval and may suggest a dysfunctional mechanism in the non-exercising bed rest group induced by two months of physical inactivity. Our results provide further support for the modulating effects of physical exercise and adverse implications of a sedentary lifestyle and bedridden patients.

1. Introduction

Physical exercise is widely suggested as an effective, low-cost, non-pharmacological strategy for maintaining and improving physical and psychological health and well-being. There is increasing evidence that regular physical activity has also considerable neurobehavioral benefits across the lifespan. Recently, particular attention has been paid to the prefrontal cortex and the hippocampal formation and their associated cognitive functions such as executive control and declarative memory (Erickson, Leckie, & Weinstein, 2014; Firth et al. 2018). For instance, cross-sectional studies in preadolescents revealed that more physically fit children showed greater hippocampal volume and greater volume was accompanied with better performance in relational memory tasks (Chaddock et al., 2010; Chaddock, Hillman, Buck, & Cohen, 2011). Likewise, there is cross-sectional evidence suggesting that elderly people engaged in habitual exercise show less degenerative symptoms and perform better in spatial memory tasks than persons of the same age not engaged in exercise (Erickson et al., 2009; Erickson et al., 2010; Bugg & Head, 2011; Szabo et al., 2011). Longitudinal training studies in rodents (van Praag, Shubert, Zhao, & Gage, 2005; Lafenetre, 2010; (Wrann et al., 2013) and humans (Erickson et al., 2011) showed that regular physical activity can increase the concentrations of (neurotrophic) growth hormones, which are critical for hippocampal plasticity, learning, and memory formation. Changes in hippocampal volume were associated with changes in brain-derived neurotrophic factor (BDNF) (Erickson et al., 2011) as well as with changes in spatial (Erickson et al., 2011) and episodic memory (Hötting et al., 2012). These data highlight the positive neurobehavioral effects associated with regular physical activity.

Likewise, it can be speculated that a sedentary life-style may have adverse effects on hippocampal plasticity, learning, and memory formation. However, previous work investigating the effects of physical inactivity or immobilization has mainly been limited to structural brain changes in patients and the elderly (Liepert, Tegenthoff, & Malin, 1995; Lissek et al., 2009; Langer, Hänggi, Müller, Simmen, & Jäncke, 2012) or focused on functional changes in other brain regions such as the frontal and parietal lobes (Yuan et al., 2016) and on the (somatosensory) motor cortex (Cassady et al., 2016; Koppelmans et al., 2018). The majority of these studies have been cross-sectional in nature, questioning the causal relationship between physical inactivity and brain plasticity. For instance, it is well-known from animal studies that

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environmental enrichment (Fabel et al., 2009) and social interaction (Djordjevic, Adzic, Djordjevic, & Radojcic, 2009; Murínová, Hlaváčová, Chmelová, & Riečanský, 2017) can also reinforce brain plasticity and memory function. These and other potential confounding factors have not been controlled for in human studies investigating the effects of physical (in)activity on the hippocampus and episodic memory formation.

To address this gap, we investigated the effects of an exercise program during two months of strict bed rest on memory-specific neuronal activity in humans using a randomized controlled trial study that standardized food, wake/sleep cycles, social interaction, and environmental enrichment. Healthy men undergoing two months of bed rest were randomly allocated to a control group and an exercise group that performed a high-intensity interval training. Both groups remained in bed rest throughout the entire study period. Using functional magnetic resonance imaging (fMRI) we determined neuronal activity during an episodic memory task that specifically targets the hippocampus and parahippocampal gyrus before and after two months of bed rest. Prolonged bed rest can be considered as a model to mimic accelerated physiological aging processes (Pavy-Le Traon, Heer, Narici, Rittweger, & Vernikos, 2007). Previous studies have shown that aging and cognitive decline are associated with hyperactive signaling of the hippocampus during episodic memory formation, which has been interpreted as a compensatory response (Hämäläinen et al., 2007; Miller et al., 2008a; Miller et al., 2008b; Nyberg et al. 2019; Yassa et al., 2010; Yassa, Mattfeld, Stark, & Stark, 2011). Consequently, we hypothesized that two months of bed rest would affect behavioral mnemonic performance and its neural basis, and that these changes were counteracted by the structured high-intensity interval training. In line with the above-mentioned studies assessing the effects of aging and cognitive decline, we expected that the maladaptive effects of prolonged physical inactivity would be evident as increases in hippocampal and parahippocampal neuronal signaling during mnemonic processing in the bed rest control group compared to the bed rest group that additionally performed the exercise program.

2. Methods

2.1. Study design

The experiment was performed as part of the European Space Agency (ESA) study "Reactive jumps in a sledge jump system as a countermeasure during long-term bed rest" (RSL). The study was conducted at the:envihab facility of the German Aerospace Center (DLR) in Cologne, Germany, and recorded on the German Clinical Trials Register (DRKS, registration number DRKS00012946, 18th of September 2017). The general study design is described elsewhere (Kramer et al., 2017a). Briefly, 23 young, healthy men underwent 60 days of six-degree head-down tilt bed rest (HDT). The baseline data collection (BDC-15 through BDC- 1) and subsequent recovery period (R+0 through R+14) lasted 15 days. On the first day of bed rest, eleven participants were randomly assigned to a high-intensity interval training (TRAIN). All participants underwent MRI scans four days prior to the bed rest commencing (BDC-4) and after 58 days of head-down tilt bed rest (HDT58). The experiment was approved by the Ethics Committee of the Northern Rhine Medical Association (Ärztekammer Nordrhein) in Düsseldorf, Germany and by the local Ethics Committee of the Charité - Universitätsmedizin Berlin, Germany. The study conformed to all standards of human research set out in the declaration of Helsinki. All participants were informed about the purpose, experimental procedures, and risks before giving their verbal and written informed consent.

2.2. Exercise training

The exercise training was performed in a supine position on a custom-built sledge jump system (Novotec Medical GmbH, Pforzheim, Germany) (Kramer et al., 2017a). All participants were familiarized with the correct jumping technique in nine 30 min sessions during the baseline data collection period. Four different training sessions consisting of varying numbers of countermovement jumps and hops were designed, and applied to TRAIN on 48 out of 60 days during the HDT phase, resulting in five to six training sessions per week (five sessions during the first two weeks of HDT and six sessions per week for the following six weeks of HDT). The force in the training device was increased gradually from 50% to 100% of participants' body weight. The total training duration of one session did not exceed more than 17 min using an average training load between 80% and 90% of the body weight. Thus, the plyometric jump training can be considered as a short-duration high-intensity training that has been shown to successfully prevent musculoskeletal and cardiovascular deconditioning caused by 60 days of bed rest (Kramer et al., 2017b; Maggioni et al., 2018). All training sessions were scheduled in the afternoon from 2 pm to 6 pm. The timing of exercise sessions was kept constant within subjects. Visual feedback for jump height and peak force were provided via a monitor. Verbal feedback was given to the participants by an exercise physiologist to ensure correct execution during each session. All participants completed all scheduled training sessions. Maximum effort was not achieved in about 6% of all familiarization and training sessions due to headache, indisposition or minor discomfort (Kramer et al., 2017a). Further details about the training protocol and adherence are provided elsewhere (Kramer et al., 2017a).

2.3. Participants and recruitment process

Subject recruitment was supported by announcements in local and nationwide newspapers, internet, radio, poster advertisement, and DLR's test participant archive. Short information was sent to all interested candidates, followed by a telephone screening. If interested, eligible participants received detailed information by email. Qualifying volunteers were invited to an information session, where the objectives, content, and risks of the experiment were explained in detail. Next, interested participants were medically and psychologically screened to ensure their compliance with all inclusion and exclusion criteria (Kramer et al., 2017a). The medical screening comprised a comprehensive anamnesis and physical examination, including an assessment of resting electrocardiogram (ECG), orthostatic tolerance, thrombosis risk, nicotine and substance abuse, the prevalence of infectious diseases, and cardiopulmonary fitness using graded exercise testing. The psychological screening was performed by a psychologist using questionnaires and a personal interview. Eligible participants underwent a dual energy X-ray absorptiometry (DEXA) scan to evaluate the bone mineral density of the femur and the lumbar vertebra column as a final criterion to be included in the study. Finally, twenty-seven volunteers passed the entire screening process and twenty-four of them were enrolled in the study. Twenty-three participants (29 ± 6 years, height: 181 ± 6 cm, weight: 77 ± 7 kg) completed the study. A detailed CONSORT flow diagram is displayed in Figure 1.

Because of medical reasons two subjects (one from each group) started their recovery on HDT 49 and HDT 50, respectively (instead of HDT60). MRI scanning for these participants was performed on the last day of their bed rest (i.e., HDT48 and HDT49 instead of HDT58). Due to an incomplete log file data set, one subject (TRAIN) had to be excluded from the data analysis. Both groups did not differ with respect to their age, height, weight, and BMI at baseline (two-tailed Student's t-test: all $Ps > 0.3$). An overview of subjects' group characteristics is displayed in Table 1.

2.4. MRI Scanning procedure

Magnetic resonance imaging was conducted on a 3 Tesla Siemens Biograph mMR (Siemens Healthcare, Erlangen, Germany), equipped with a 16-channel head coil. Functional images were acquired using a T2-weighted echo planar imaging (EPI) sequence sensitive to blood oxygen

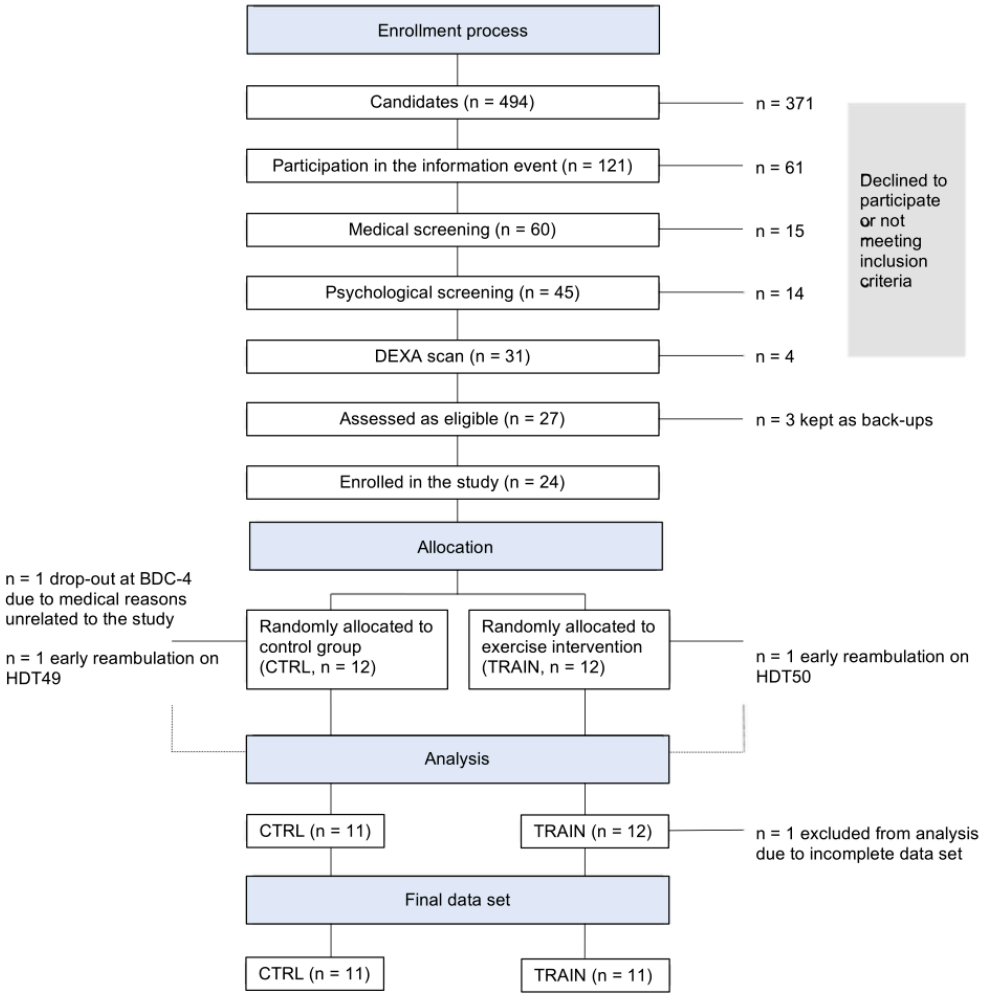

Fig. 1. CONSORT flow diagram. Overview of recruiting, enrollment, and analysis process.

Table 1
Subjects' group characteristics at baseline.*

Characteristic	CTRL (n = 11)	TRAIN (n = 11)	t_{20}	P
Age [years]	28.2 (5.7)	30.8 (6.2)	1.02	0.318
Height [cm]	180.6 (5.0)	181.6 (6.8)	0.39	0.699
Body Mass [kg]	76.2 (8.0)	77.9 (6.6)	0.54	0.593
BMI [kg/m^2]	23.4 (2.0)	23.6 (1.9)	0.24	0.812

* Data are means (SD); BMI, Body Mass Index; CTRL, bed rest control group; TRAIN, exercising bed rest group; *t*, t-statistics; *P*, p-value.

level dependent (BOLD) contrast (36 axial slices, interleaved slice order, time to repeat (TR) = 2000 ms, time to echo (TE) = 30 ms, field of view (FoV) read = 216 mm, FoV phase = 100%, flip angle = 80°, slice thickness = 3 mm, distance factor = 20%, voxel size: 3 mm × 3 mm × 3 mm). For anatomical reference, a three-dimensional volumetric T1-weighted Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE) sequence was acquired in a sagittal plane with the following parameters: voxel size: 1 mm × 1 mm × 1 mm; TR = 2500 ms, TE = 4.82 ms, inversion time = 1100 ms, FoV read = 256 mm, FoV phase = 100%, flip angle = 7°, and bandwidth = 140 Hz/Px. Scanning was always performed before noon between 8 am and 12 pm.

2.5. *fMRI paradigm*

Episodic memory was assessed using a continuous memory recognition task that was originally proposed by Kirwan & Stark (2007) and that was shown to reliably target the hippocampus (Bakker, Kirwan, Miller, & Stark, 2008; Kirwan et al., 2012; Ally, Hussey, Ko, & Molitor, 2013). A schematic overview of the fMRI paradigm is displayed in Fig. 2. Images of different objects were presented to the participants via a mirror system mounted on top of the head coil. Each picture was shown for 2000 ms with a randomized intertrial interval (ITI) of 2000 to 4000 ms. For each picture, participants had to indicate via a button press whether

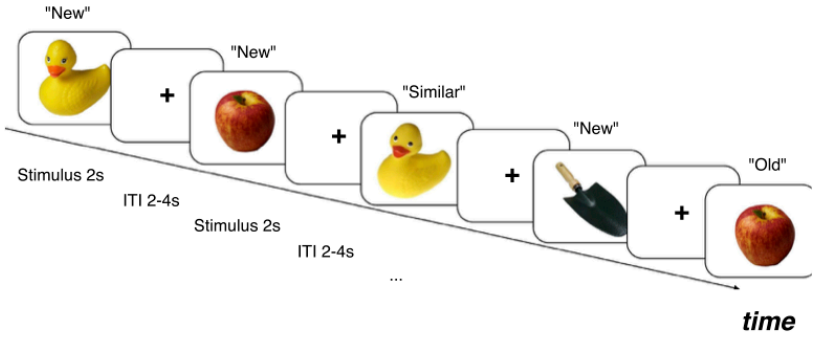

Fig. 2. fMRI paradigm for pattern separation task. A picture was presented for 2 s followed by an intertrial interval (ITI) of 2 to 4 s. Quotation marks above the picture indicate the correct response.

the presented object was "new" (novel condition), "old" (repetitive condition), or "similar", though not identical to an object shown earlier in the run (lure condition). The experiment consisted of 216 trials administered in two blocks, each containing 108 items (16 similar pairs, 16 identical pairs and 44 unrelated novel stimuli) with an overall duration of approximately six minutes per run. To avoid that participants remembered the objects from pre- to posttest, one of two sets of stimuli were either presented on BDC-4 or HDT58. The order of the two sets was selected in a randomized counterbalanced fashion. Picture presentation and timing was controlled using Presentation® software (Version 18.1, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com).

2.6. Behavioral analysis

For each group and time point separately, the number of each stimulus type (novel, lure, repetition) in accordance with participants' individual response (new, similar, old) was extracted from the data log file. We then calculated the percentage for each response type relative to the total number of trial type (i.e., number of correctly identified repeated stimuli as old relative to the total number of repeated stimuli). In order to distinguish between signal and noise that corrects for a possible response bias, we also determined separation bias (Yassa et al., 2010), also referred to as the lure discrimination index (Stark, Stevenson, Wu, Rutledge, & Stark, 2015) and recognition memory performance (Stark, Yassa, Lacy, & Stark, 2013).[1] The separation bias score was assessed as the percent of lure trials correctly identified as "similar" (Lure Correct Rejection) minus the percent of novel trials endorsed as "similar". This approach corrects for a possible response bias toward exhibiting a tendency for the use of similar responses (Yassa et al., 2010). Recognition memory performance was operationalized as the percent of repetition trials correctly identified as "old" (Hits) minus the percent of novel trials endorsed as "old" (Stark et al., 2013). The behavioral response according to each trial type as well as the separation bias and recognition memory scores were subjected to mixed linear models to quantify the effects of *Time* (BDC-4, HDT58) and *Group* (CTRL, TRAIN), and their interaction. Details of the statistical model are provided in section 2.8 below.

[1] Typically, d' prime is used as a sensitivity index to distinguish between signal and noise that corrects for a possible response bias in two-item response paradigms. For instance, in a yes/no discrimination paradigm giving the same answer for all trials yields 100% correct for one item, and 0% for the other (Pallier, 2002). The current task, however, was characterized by a three-choice serial reaction time task, requiring a slightly different analysis approach, taking into account three alternative responses. We here follow the suggestions to determine separation bias and recognition memory score for the pattern separation paradigm as outlined by Stark and colleagues (Stark, Yassa, Lacy, & Stark, 2013; Stark, Stevenson, Wu, Rutledge, & Stark, 2015; Yassa et al., 2010).

2.7. fMRI Analyses

2.7.1. Image preprocessing

fMRI data were preprocessed and analyzed using SPM12 software (Wellcome Department of Cognitive Neurology, London, UK) running on Matlab R2015b. The first four volumes of all EPI series were excluded from the analysis to allow the magnetization to reach a dynamic equilibrium. Data processing started with slice time correction and realignment of the EPI datasets. A mean image of EPI volumes was created to which individual volumes were spatially realigned by means of rigid body transformations. The individual structural image of each subject was co-registered with the mean image of the respective EPI series. The structural images were normalized to the Montreal Neurological Institute (MNI) template and normalization parameters were applied to the EPI images to ensure an anatomically informed normalization. A commonly applied filter of 8 mm FWHM (full-width at half maximum) was used to smooth the images. Low-frequency drifts in the time domain were removed by modelling the time series for each voxel by a set of discrete cosine functions to which a cut-off of 128 seconds was applied.

2.7.2. Subject-level analyses

Region of interest model. The subject-level statistical analyses were performed using general linear models (GLM) within the SPM-framework. The model was based on the first-level model reported by Kirwan and Stark (2007) using the following conditions: 1) *Hits* (repeated stimuli correctly called "old"; 2) *Lure Correct Rejection* (lure stimuli correctly identified as "similar"); 3) *Lure False Alarms* (lure stimuli called "old"); 4) *Miss* (repeated or lure stimuli called "new"); 5) *Subsequent Hits* (first time presentation of a repetition stimuli that was later correctly identified as "old"); 6) *Subsequent Lure Correct Rejection* (first time presentation of a lure stimuli that was later correctly identified as "similar"); 7) *Subsequent Lure False Alarms* (first time presentation of a lure stimuli that was later incorrectly identified as "old"); 8) *Subsequent Misses* (first time presentation of a repetition or lure stimuli that was later incorrectly labeled as "new"); 9) *Foils* (stimuli that have only been shown once and not in the same or similar way again and have been classified as "new"); and 10) *Other* (foils and first presentations that were incorrectly labeled as "old" or "similar"). Vectors of onsets for each of the above-mentioned event types for each participant and each point in time were convolved with the canonical hemodynamic response function (HRF) and the temporal derivative. Both session runs that have been administered at one point in time were modeled in the same GLM. Furthermore, the six movement regressors obtained from the realignment step were

entered into the GLM. After model estimation, this model was used for the region of interest analysis (ROI).

To verify if the regions of the medial temporal lobe were engaged in the task, we additionally computed a contrast on our first-level GLM from the ROI analysis were the average activity in *Hits* is greater than the average in *Lure Correct Rejection* and *Foils* (*Hits* > *Lure CR* = *Foils*) and a contrast where average activity in *Lure Correct Rejection* is greater than the average activity in *Hits* and *Foils* (*Lure CR* > *Hits* = *Foils*). These contrasts were computed for all participants during their baseline assessment (BDC-4) before they were assigned to any intervention. The resulting contrast images were used for group-level statistics within the SPM-framework.

Whole-brain model. For whole-brain data we modeled a second GLM on a subject-level, but limited the analyses to conditions with correct responses, i.e., *Foils, Hits,* and *Lure Correct Rejections* (Pidgeon & Morcom, 2016). We computed *t*-contrasts for pattern separation and pattern completion as follows: Pattern separation was defined as the contrast where average activity in *Lure Correct Rejection* is the same as in *Foils* and greater than the average activity in *Hits* (*Lure CR* = *Foils* > *Hits*), and pattern completion was defined as the contrast where average activity in *Foils* was greater than in *Hits* and *Lure Correct Rejection* (*Foils* > *Hits* = *Lure CR*) (Pidgeon & Morcom, 2016). The resulting contrast images were used for further whole brain analysis on a group level.

2.7.3. Group-level analyses

Region of interest analysis. Anatomical regions of interests were determined a priori based on 1) regions that have been shown to be vulnerable to physical exercise, and 2) regions that have been reported previously to be involved in the task that we used. Previous work on the effects of physical exercise suggests that the hippocampus is significantly affected by regular exercise (Erickson et al., 2011; Firth et al., 2018). The pattern separation paradigm used in the present study distinctly activates the hippocampus and parahippocampal gyri (Kirwan & Stark, 2007; Bakker et al., 2008). Hence, we retrieved BOLD signal change in bilateral hippocampus and parahippocampal gyrus from the Automated Anatomical Labeling (AAL) Atlas (Tzourio-Mazoyer et al., 2002). This subject-level design matrix was used to extract the mean percent signal changes for each subject, each condition, and each point in time. Given that blood oxygenation peaks 4 to 6 seconds post-stimulus (Poldrack, Mumford & Nichols, 2011; Chen, Shen & Truong, 2016), we extracted the mean percent signal change covering a time window of 4 to 6 seconds after stimulus onset for each of the four regions of interest (left and right hippocampus and left and right parahippocampal gyrus) using the MarsBaR toolbox (http://marsbar.sourceforge.net/) (Brett, Anton, Valabregue, & Poline, 2002). Differences in mean percent signal change of hippocampal and parahippocampal activation were analyzed using mixed linear models (see also statistical models in section 2.8 for details). To validate the paradigm on the group-level, the contrast images for the contrasts *Hits* > *Lure CR* = *Foils* and *Lure CR* > *Hits* = *Foils* that had been computed across all participants during the baseline assessment prior to the intervention (BDC-4) were subjected to a one-sample *t*-test within the SPM-framework to verify which regions were engaged during the retrieval of repetitive and lure stimuli. The results were corrected using a family wise error rate (FWE).

Whole-brain analyses. The contrast images of the pattern separation and completion contrasts that had been acquired on a subject-level were used in a flexible factorial design within the SPM framework with *Subject* as a random factor, and *Time* and *Group* as fixed factors. Contrasts for main and interaction effects were computed and results were corrected for multiple comparisons using a family wise error rate (FWE).

2.8. Statistical analysis

Behavioral data and mean percent BOLD signal change for our *a priori* regions of interest, i.e., bilateral hippocampus and parahippocampal gyrus were analyzed by linear mixed models. To assess the effects of bed rest and the exercise intervention on percent responses classified as new, similar or old, we performed mixed models with *Group* (CTRL, TRAIN), *Time* (BDC-4, HDT58), and their interaction as fixed factors, and *Subject* as a random factor. The inclusion of *Stimulus Type* (novel, lure, repetition) as an additional factor did not allow precise estimations of the variance-covariance matrices. Hence, we ran separate mixed models for each stimulus (novel, lure, repetition) and response type (new, similar, old). Mean percent BOLD signal change was assessed for hippocampus and parahippocampal gyrus in two separate models. In each model, we entered *Time* (BDC-4, HDT58), *Group* (TRAIN, CTRL), *Laterality* (Left, Right), *Condition* (Encoding, Retrieval), and *Stimulus Type* (Lure, Repetition). Covariance matrices were determined by restricted maximum likelihood (REML) estimation. P-values were obtained by using Satterthwaite's approximation for denominator degrees of freedom. Pre-planned contrasts were used to quantify the interaction between *Time* and *Group* by *Laterality* crossed with *Condition* and *Stimulus Type* for BOLD signal change. Contrasts were adjusted using a false discovery rate (FDR) procedure treating each region of interest (left and right hippocampus and parahippocampal gyrus) as one family of four comparisons each (two comparisons for *Condition* x two comparisons for *Stimulus Type*) (Benjamini & Hochberg, 1995). Effect sizes were reported as Cohen's *d* and 95% confidence intervals. For imaging data, a false coverage statement rate (FCR) was computed using a Matlab script (Groppe, 2020) to construct multiple comparison corrected confidence intervals that correspond to the FDR-adjusted p-values (Benjamini & Yekutieli, 2005). The level of significance was set at = 0.05 (two-sided) for all tests. All statistical analyses and graphical illustrations were carried out using the software package R version 3.5.2 (R Core Team, 2018).

3. Results

3.1. Validation of the fMRI paradigm

All participants demonstrated high accuracy in correctly identifying novel (> 93%) and repetitive stimuli (> 90%), whereas lure stimuli were harder to classify correctly (between 43% to 51% for BDC-4). A detailed summary of the descriptive statistics is provided in the Supplementary Material (Table S1). To verify which regions were engaged in the paradigm, we assessed brain activations during the contrasts *Hits* > *Lure CR* = *Foils* and *Lure CR* > *Hits* = *Foils* at the first point in time (BDC-4) for all participants (n = 22). Clusters that were significantly activated for the contrast *Hits* > *Lure CR* = *Foils* were the left fusiform gyrus, left hippocampus, left MTG (for all 3 clusters $P_{FWE\text{-}corr.} = 0.033$), and bilateral precuneus ($P_{FWE\text{-}corr.} < 0.001$) as well as bilateral cerebellum ($P_{FWE\text{-}corr.} = 0.029$ for right and $P_{FWE\text{-}corr.} = 0.002$ for left cerebellum). For the contrast *Lure CR* > *Hits* = *Foils*, we observed significant activations in bilateral precuneus ($P_{FWE\text{-}corr.} < 0.001$), left hippocampus ($P_{FWE\text{-}corr.} = 0.042$), and bilateral cerebellum ($P_{FWE\text{-}corr.} = 0.027$ for right and $P_{FWE\text{-}corr.} = 0.026$ for left cerebellum). Both, the behavioral performance and the regions activated at BDC-4 are very similar to the results reported previously confirming the validity of the paradigm (Hämäläinen et al., 2007; Kirwan & Stark, 2007; Pidgeon & Morcom, 2011).

3.2. Behavioral findings

Figure 3 shows frequency of the responses given to each stimulus type for the contrast between HDT58 and BDC-4. Both groups showed a significant improvement in the discrimination of lure items by identifying them more often as "similar" and less as "old" on HDT58 (all *P*s < 0.05). Numerically, TRAIN also improved in the recognition of repetition trials ($t_{20} = 0.46$, $P = 0.654$, $d = 0.19$, [-0.65, 1.03]), whereas CTRL showed a small decrease from HDT58 to BDC-4 ($t_{20} = -0.76$, $P = 0.457$, $d = -0.32$, [-1.16, 0.52]). There was neither a significant main effect for *Group* nor a *Group* x *Time* interaction in any of the behavioral

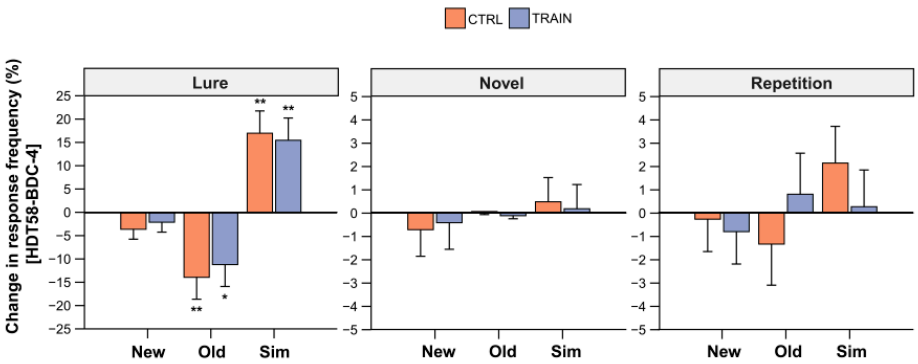

Fig. 3. Changes in response frequency from baseline (HDT58 vs. BDC-4) by *Stimulus Type* (lure, novel and repeated stimuli), *Group* (TRAIN, CTRL), and *Response* (items classified as new, old, or similar). Data are marginal means and standard errors. CTRL, bed rest control group; TRAIN, exercising bed rest group. N = 11 for each group respectively. * $P < 0.05$, ** $P < 0.01$ compared to BDC-4.

conditions (all *Ps* > 0.124). Detailed statistical results are provided in Supplementary Material (Table S2 and S3).

Results for the separation bias and the recognition memory score were similar to changes observed for discriminating lure and repetition trials (see Supplementary Material Figure S1). We also observed an increase in the separation bias score in both groups ($F_{1,20} = 26.16$, $P < 0.001$ for *Time*). This is in line with the improvement in discriminating lure trials, given that the separation bias score was calculated as the difference between the probability of identifying a lure item as "similar" and the probability of identifying a novel foil item as "similar". Numerically, TRAIN showed a higher and CTRL a lower recognition memory score that, however, did not reach statistical significance (*Group* x *Time*, $F_{1,20} = 0.89$, $P = 0.358$). Detailed statistical results are provided in Supplementary Material Table S4.

3.3. fMRI results

3.3.1. Region of interest analysis

After two months of bed rest, increases in BOLD signal were observed in CTRL for all mnemonic conditions, whereas the signal in TRAIN decreased (Fig. 4). Pre-planned contrasts revealed that the decrease in BOLD signal within TRAIN was significant for five out of eight conditions in the left hemisphere (all *Ps* < 0.05). A detailed summary of the simple effects of *Time* by *Laterality* (Left, Right) and *Condition* (Encoding, Retrieval) and *Stimulus Type* (Lure, Repetition) for each group separately and for all main and interaction effects of the multilevel analysis is provided in Supplementary Material Table S5, S6, and S7. We also observed a significant interaction between *Group* and *Time* in the left hemisphere for the retrieval process of lure stimuli (hippocampus: $P = 0.035$, $d = -1.12$ [-2.27, 0.04]); parahippocampal gyrus: $P = 0.012$, $d = -1.23$ [(-2.14, -0.30]). Furthermore, a significant interaction of *Group* x *Time* during correct encoding and retrieval of repetition stimuli was found in the left parahippocampal gyrus ($P = 0.019$, $d = -1.01$[-1.89, -0.11]; $P = 0.018$, $d = -1.05$ [-1.93, -0.14], respectively). A nearly significant interaction was observed for the left hippocampus during encoding of lure items ($P = 0.054$, $d = -0.95$ [-2.07, 0.19]) and for the right parahippocampal gyrus during retrieval of lures ($P = 0.056$, $d = -1.05$). There were no other significant main or interaction effects (all *Ps* > 0.215). Details of these analysis are provided in Supplementary Material Table S8.

3.3.2. Whole-brain analysis

Greater activation on HDT58 compared to BDC-4 for the pattern separation contrast [BDC-4 (*Lure CR* = *Foils* > *Hits*)] vs. [HDT58 (*Lure CR* = *Foils* > *Hits*)] was observed in the right occipital pole (R OCP), right middle temporal gyrus (R MTG), frontal pole, right fusiform gyrus (R FuG), and left inferior temporal gyrus (L ITG) for CTRL ($P < 0.001$, clusterwise FWE-corrected ($P < 0.05$)). Notably, only the right OCP survived the FWE-cluster correction (Fig. 5). Higher activation was also seen in the right superior parietal lobule in TRAIN, but did not reach the level of significance. There was no *Group* x *Time* interaction for the pattern separation contrast.

Regions showing decreased activation for the pattern completion contrast [BDC-4 (*Foils* > *Hits* = *Lure CR*)] vs. [HDT58 (*Foils* > *Hits* = *Lure CR*)] involved bilateral precentral gyrus, bilateral insular cortex, and right middle temporal gyrus for CTRL and left superior temporal gyrus (L STG) and left occipital fusiform gyrus (L OFuG) for TRAIN. Only the bilateral precentral gyrus survived FWE-cluster correction. A *Group* x *Time* interaction that did not reached statistical significance was observed in the right precentral gyrus. A detailed overview of the regions that showed changes in the pattern separation and completion contrast from BDC-4 to HDT58 and exceeded the threshold of 20 voxels is provided in the Supplementary Material Table S9 and S10.

4. Discussion

We investigated the effects of long-term bed rest on episodic memory performance and its neural basis and whether a high-intensity jump training can mitigate the effects of physical inactivity on the neural underpinnings of memory functioning. After two months of bed rest, we found increases in BOLD signal during memory encoding and retrieval in the hippocampal formation in CTRL compared to TRAIN, suggesting a modulating effect of the exercise intervention. The strongest effects of exercise were observed in the left hemisphere. This is in line with recent research summarizing the effects of regular physical activity on the hippocampus. In a meta-analysis of 14 longitudinal studies, Firth et al. (2018) reported significantly larger effects for the left hippocampus (Hedge's *g* [95% CI] = 0.265 [0.090, 0.441], $P = 0.003$) compared to the right hippocampus (Hedge's *g* [95% CI] = 0.164, [-0.010, 0.339], $P = 0.065$).

Increased brain activity during pattern separation has been observed in the elderly compared to young adults (Yassa et al., 2011), as well as in patients with mild cognitive impairment (MCI) compared to healthy controls (Hämäläinen et al., 2007; Yassa et al., 2010). Bed rest is a classical model to simulate some of the physiological adaptations associated with spaceflight (Pavy-Le Traon et al., 2007). Given that the physiological responses to spaceflight reflect an accelerated aging process (McGuire et al., 2001; Vernikos & Schneider, 2010), bed

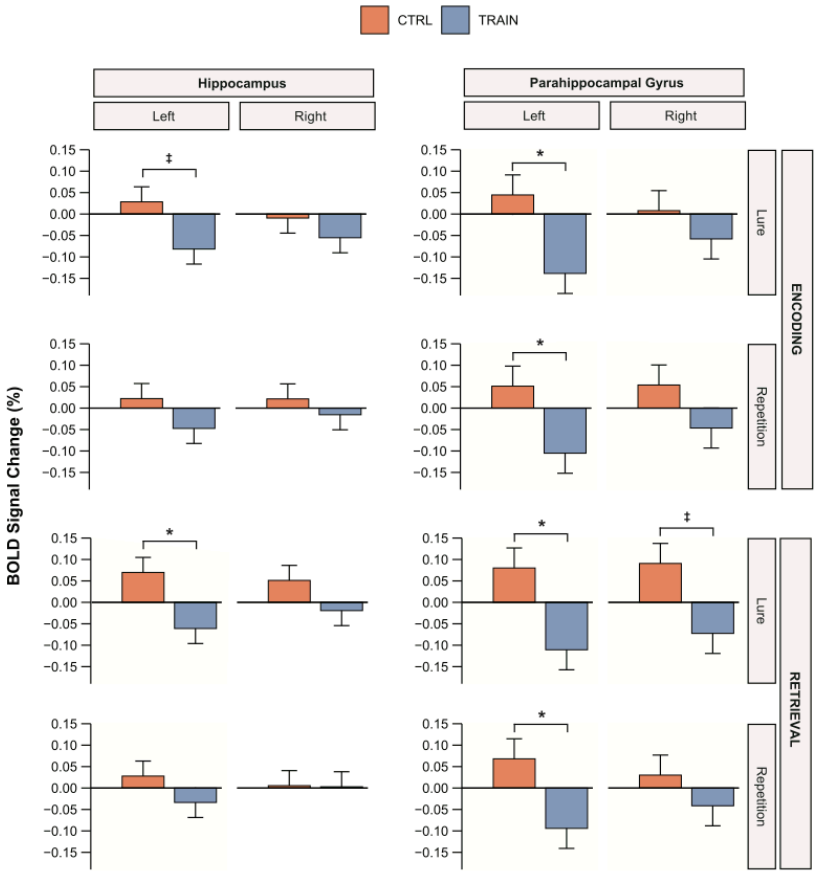

Fig. 4. BOLD signal change during memory encoding and retrieval of lure and repetition stimuli for bilateral hippocampus and parahippocampal gyrus. Data are marginal means and standard errors for the interaction of *Group* (TRAIN, CTRL) x *Time* (BDC-4, HDT58) by *Laterality* (Left, Right) crossed with *Condition* (Encoding, Retrieval) and *Stimulus Type* (Lure, Repetition). CTRL, bed rest control group; TRAIN, exercising bed rest group. N = 11 for each group respectively. * $P < 0.05$, ‡ interaction was close to statistical significance with $P = 0.054$ for left hippocampus during encoding of lure stimuli and $P = 0.056$ for right parahippocampal gyrus during retrieval of lure stimuli.

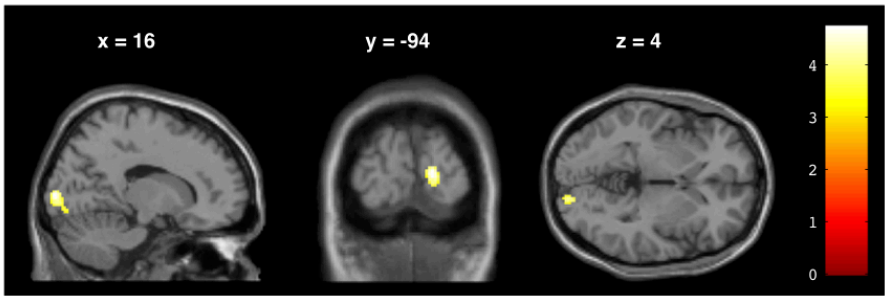

Fig. 5. Right occipital pole showing increased activation during pattern separation in CTRL. SPMs for the contrast [BDC-4 (*Lure CR = Foils > Hits*)] vs. [HDT58 (*Lure CR = Foils > Hits*)] averaged over 11 CTRL subjects mapped onto an MNI template ($P < 0.001$, clusterwise FWE-corrected ($P < 0.05$)).

rest can also be considered as a unique model to better understand the effects of premature physiological aging. In this regard, our findings of increased brain activation in CTRL could be interpreted as a consequence of bed rest-induced accelerated aging. This is also supported by findings from Miller and colleagues (Miller et al., 2008a), showing a very similar response in the elderly. Specifically, they observed greater hippocampal activity during the encoding process in low-performing older adults and explained this phenomenon as a compensatory response (Miller et al., 2008a). In another study Miller and colleagues also demonstrated that greater hippocampal activation during memory encoding predicted the rate of cognitive decline (Miller et al., 2008b). Additional evidence comes from Yassa et al. (2010) who found an inverse relationship between hyperactivity in the dentate gyrus and CA3 region and behavioral performance, suggesting that the increase in brain activity resulted from dysfunctional encoding mechanisms. Conflicting results have been reported in a precedent study by Small and colleagues (Small, Perera, DeLaPaz, Mayeux, & Stern, 1999) who found reduced activation in all hippocampal subfields in Alzheimer patients compared to healthy elder controls. Sperling speculated that hyperactivation occurs in very early stages of MCI as a compensatory response to maintain memory performance but at a later stage the hippocampus fails, resulting in decreased activation (Sperling, 2007). In a longitudinal study Nyberg and colleagues (2019) have observed both during encoding, an age-related hypoactivity in the anterior hippocampus, and hyperactivity in the anterior and posterior hippocampus in elderly having lower memory performance and a higher dementia risk. The authors suggested that hippocampal hyperactivity is not a response per se to normal aging but rather a pathological sign of neurocognitive disorders (Nyberg et al. 2019). The same group also assumed that hippocampal atrophy and memory decline induces functional reorganization in the prefrontal cortex (Pudas et al., 2018) and elevated functional connectivity between PFC and anterior hippocampus (Nyberg et al. 2019).

In the present study, differences in brain activity between groups were not paralleled by changes in behavioral performance. Participants most often identified novel, lure, and repetition stimuli correctly as new, similar, and old showing the highest accuracy in novel and repetition items. Lure items were harder to classify and only correctly identified in 43% to 66% of all lure trials. Very similar performances on novel, lure and repetitive trials have been reported in previous studies (Hämäläinen et al., 2007; Kirwan & Stark, 2007) confirming the validity of the paradigm in the present study. Notably, we observed an improvement in the ability of discriminating lure trials on HDT58 compared to BDC-4 in both groups that may be the result of additional test-enhanced learning effects that occurred independently of the intervention. It can only be speculated whether the discrepancy between neuronal activity and mnemonic performance is due to a reduced neuronal efficiency in CTRL (i.e., that maintaining behavioral performance requires a higher neural demand) or that bed rest induced dysfunctional mechanisms in neuronal coupling, which were counteracted by the exercise intervention. In addition to our *a priori* hypothesis anticipating changes in hippocampus and parahippocampal gyrus, we also explored whole-brain BOLD signal changes during pattern separation and completion from BDC-4 to HDT58. We observed a significant increase in the right occipital pole during pattern separation in participants that did not undergo the training intervention. Occipital, parietal, and temporal regions have been reported previously to contribute to pattern separation (Pidgeon & Morcom, 2016) and memory retrieval (Jonker et al., 2018). It is also possible that these activations reflect other processes associated with the encoding of the stimuli. For instance, Sestieri, Shulman, & Corbetta (2017) noted that such activations could be related to perceptual attention. Our data also suggested a stronger decrease in CTRL for bilateral precentral gyrus. However, the interaction between *Group* and *Time* was not significant. It is possible that this activation is likely to be attributed to bed rest (Cassady et al., 2016). Cassady and colleagues reported that the intrinsic connectivity contrast (ICC) of the precentral gyrus is increased after 70 days of bed rest (Cassady et al., 2016). We therefore assume that the observed cluster is unrelated to our fMRI paradigm and a result of prolonged bed rest per se.

Key strengths of this study are the highly-standardized conditions and environment of the experimental and control group. In contrast to previous ambulatory training studies (Erickson et al., 2011; Ruscheweyh et al., 2011; Hötting et al., 2012), we were able to standardize various critical factors that are known to affect neurobehavioral measures, including the social environment, leisure time activities, nutrition, sleep, and day and night cycles. Over three months, including the 60 days of bed rest, sleep, diet, light exposure, environmental conditions, and physical activity were strictly regulated and standardized (Kramer et al., 2017a). Social interactions were limited to staff, other participants, and individual personal phone calls only. Thus, the differences in neuronal activity observed in the study can likely be exclusively attributed to the result of the exercise intervention. It should also be noted that the exercise intensity and target population of the current project differed compared to previous studies.

Our exercise group followed a short but intensive jump training protocol and completed 48 sessions within two months. For example, this was the same amount of sessions over a course of six months in the study by Hötting et al. (2012). Moreover, a large part of the existing body of research focuses on older adults with a mean age >60 years (e.g., Erickson et al., 2011; Ruscheweyh et al., 2011; Jonasson et al., 2016). Only very few studies with a similar cohort following a comparable study design have been reported so far and none of these studies particularly targeted the function of the hippocampus (Koppelmans et al., 2013; Rao et al., 2014; Zhou et al., 2014; Yuan et al., 2016). In these studies, 45 days of bed rest led to altered functional connectivity in the left anterior insula and dorsal anterior cingulate cortex (Zhou et al., 2014) as well as to greater activation in the ventromedial prefrontal cortex during risky decision making (Rao et al., 2014). Another research group reported increased brain activity in frontal and parietal regions that were accompanied with slower reaction times (Yuan et al., 2016) and detrimental effects on functional connectivity in motor and somatosensory brain areas after 70 days of bed rest (Cassady et al., 2016; Koppelmans et al., 2017; Koppelmans et al., 2018). The authors concluded from the observed increased brain activation that more neurocognitive control is required during bed rest for dual-task execution (Yuan et al., 2016). The results of the present study confirm these findings for episodic memory and its neural basis, and provide novel insights into the effects of physical activity on mitigating the effects of bed rest on brain function.

4.1. Limitations

Although the study was highly standardized, our findings are subject to a few limitations. The overall experimental protocol was defined by the study sponsor, including inclusion and exclusion criteria as well as sample size. These restricted criteria have resulted in a highly selective sample of young, healthy men. Accordingly, caution must be applied with respect to the generalizability of the findings to women, the aging population, and patients. Further research is needed to investigate bed rest induced changes in mnemonic processing and their neural basis in different populations. We also acknowledge that the present study lacks a non-resting control group. Albeit, the challenges in controlling for diet, sleep, social contacts, and physical activity levels in ambulatory controls, such data could also provide important information to better understand the effects of long-duration immobilization.

5. Conclusion

The current study assessed the effects of long-term bed rest on episodic memory and its neural correlates and the efficacy of a regular high-intensity exercise to mitigate adverse neurobehavioral effects. With the same mnemonic performance, we found an elevated BOLD

signal in the non-exercising bed rest group compared to the exercising bed rest group. It cannot be conclusively decided whether this is a compensatory response or the result of an underlying dysfunctional mechanism. Our findings show, however, that high-intensity exercise modulates neuronal activity and may counteract hyperactive signaling in the hippocampal formation. Further research is needed to elucidate sex-specific effects of the high-intensity exercise program on hippocampal activation, and explore the potential of these programs to preserve brain function in the aging population.

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CRediT authorship contribution statement

Anika Friedl-Werner: Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Katharina Brauns:** Project administration, Investigation, Data curation, Writing - review & editing. **Hanns-Christian Gunga:** Writing - review & editing. **Simone Kühn:** Methodology, Software, Formal analysis, Writing - review & editing. **Alexander C. Stahn:** Conceptualization, Supervision, Project administration, Funding acquisition, Methodology, Formal analysis, Visualization, Writing - original draft, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2020.117359.

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Supplementary Materials

Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest

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1 Supplementary Tables

Table S1. Descriptive statistics of the behavioral response by stimulus type.*

Stimuli	Response	BDC-4		HDT58	
		CTRL	TRAIN	CTRL	TRAIN
Novel	New	96.77 (3.8)	94.32 (7.5)	96.05 (3.9)	93.90 (6.8)
Novel	Similar	2.75 (3.6)	4.96 (7.5)	3.23 (3.9)	5.14 (6.4)
Novel	Old	0.30 (0.5)	0.36 (0.5)	0.36 (0.5)	0.24 (0.6)
Lure	New	12.73 (8.9)	10.00 (6.3)	9.09 (10.0)	7.88 (4.0)
Lure	Similar	43.33 (25.6)	50.91 (20.1)	60.30 (18.0)	66.36 (13.8)
Lure	Old	42.12 (21.4)	36.67 (16.7)	28.18 (18.9)	25.45 (13.4)
Repetition	New	4.01 (6.9)	2.14 (1.4)	3.74 (3.0)	1.34 (1.5)
Repetition	Similar	2.94 (4.4)	5.08 (3.5)	5.08 (4.8)	5.35 (5.4)
Repetition	Old	92.24 (9.5)	92.5 (4.4)	90.91 (7.3)	93.32 (6.0)
Missed responses		0.51 (0.6)	0.63 (0.9)	0.63 (1.1)	0.55 (1.2)

*Data are means and their standard deviation. BDC-4, baseline data collection 4 days prior to bed rest; HDT58, 58 days of head-down tilt bed rest, CTRL, bed rest control group; TRAIN, exercising bed rest group.

Table S2. Mixed-models assessing the effects of *Group*, *Time*, and their interaction on behavioral performance (new, similar, old) according to each stimulus type (novel, lure, repetition).*

Effect	Stimuli	Response	df_1	df_2	*F*	*P*
Group	Novel	New	1	20	0.98	0.333
Time	Novel	New	1	20	0.50	0.489
Group x Time	Novel	New	1	20	0.03	0.855
Group	Novel	Similar	1	20	0.83	0.374
Time	Novel	Similar	1	20	0.20	0.659
Group x Time	Novel	Similar	1	20	0.04	0.841
Group	Novel	Old	1	20	0.02	0.877
Time	Novel	Old	1	20	0.11	0.748
Group x Time	Novel	Old	1	20	0.96	0.340
Group	Lure	New	1	20	0.46	0.506
Time	Lure	New	1	20	3.67	0.070
Group x Time	Lure	New	1	20	0.25	0.620
Group	Lure	Similar	1	20	0..77	0.390
Time	Lure	Similar	1	20	23.05	<0.001
Group x Time	Lure	Similar	1	20	0.05	0.825
Group	Lure	Old	1	20	0.36	0.557
Time	Lure	Old	1	20	14.36	0.001
Group x Time	Lure	Old	1	20	0.17	0.689
Group	Repetition	New	1	20	2.58	0.124
Time	Repetition	New	1	20	0.30	0.592
Group x Time	Repetition	New	1	20	0.07	0.788
Group	Repetition	Similar	1	20	0.57	0.459
Time	Repetition	Similar	1	20	1.17	0.292
Group x Time	Repetition	Similar	1	20	0.71	0.411
Group	Repetition	Old	1	20	0.24	0.631
Time	Repetition	Old	1	20	0.05	0.832
Group x Time	Repetition	Old	1	20	0.74	0.401

*Mixed-models were performed separately for each stimulus type and behavioral response. *Time* (BDC-4, HDT58) and *Group* (CTRL, TRAIN) were treated a fixed factors and *Subject* as a random factor. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom; *F*, F-statistics, *P*, p-value.

Table S3. Contrasts comparing the effects of *Time* on behavioral performance (new, similar, old) according to each stimulus type (novel, lure, repetition) and each group.*

Group	Stimuli	Response	*df*	*t-ratio*	*P*	*Effect Size (95% CI)*
CTRL	Novel	New	20	-0.63	0.536	-0.27 (-1.10, 0.57)
TRAIN	Novel	New	20	-0.37	0.717	-0.16 (-0.99, 0.68)
CTRL	Novel	Similar	20	0.46	0.65	0.20 (-0.64, 1.03)
TRAIN	Novel	Similar	20	0.17	0.865	0.07 (-0.76, 0.91)
CTRL	Novel	Old	20	0.46	0.650	0.20 (-0.64, 1.03)
TRAIN	Novel	Old	20	-0.92	0.367	-0.39 (-1.23, 0.46)
CTRL	Lure	New	20	-1.71	0.103	-0.73 (-1.59, 0.14)
TRAIN	Lure	New	20	-1.10	0.330	-0.43 (-1.27, 0.43)
CTRL	Lure	Similar	20	3.55	0.002	1.52 (0.54, 2.46)
TRAIN	Lure	Similar	20	3.24	0.004	1.38 (0.43, 2.30)
CTRL	Lure	Old	20	-2.97	0.008	-1.27 (-2.18, -0.33)
TRAIN	Lure	Old	20	-2.39	0.027	-1.02 (-1.90, -0.11)
CTRL	Repetition	New	20	-0.19	0.849	-0.08 (-0.92, 0.76)
TRAIN	Repetition	New	20	-0.58	0.570	-0.25 (-1.08, 0.60)
CTRL	Repetition	Similar	20	1.36	0.190	0.58 (-0.28, 1.43)
TRAIN	Repetition	Similar	20	0.17	0.867	0.07 (-0.76, 0.91)
CTRL	Repetition	Old	20	-0.76	0.457	-0.32 (-1.16, 0.52)
TRAIN	Repetition	Old	20	0.46	0.654	0.19 (-0.65, 1.03)

*_Time_ (HDT58, BDC-4). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. *df*, degrees of freedom, *P*, p-value; Effect size is Cohen's *d* and the corresponding 95% confidence interval (95% CI).

Table S4. Mixed-models assessing the effects of *Group*, *Time*, and their interaction on separation bias and recognition memory score.*

Score	Effect	*df1*	*df2*	*F*	*P*
Separation bias	Group	1	20	0.33	0.570
	Time	1	20	26.16	< 0.001
	Group x Time	1	20	0.04	0.847
Recognition memory	Group	1	20	0.25	0.624
	Time	1	20	0.04	0.850
	Group x Time	1	20	0.89	0.358

*Mixed-models were performed separately for each score. *Time* (BDC-4, HDT58) and *Group* (CTRL, TRAIN) were treated a fixed factor and *Subject* as a random factor. n = 11 for each group respectively. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom; *F*, F-statistics, *P*, p-value.

Table S5. Contrasts of ROI analyses assessing the effects of *Time* (BDC-4 vs. HDT58) on BOLD signal change in bilateral hippocampus and parahippocampal gyrus within each group.*

Group	Region	Laterality	Condition	Stimulus Type	*df*	*t-ratio*	*P*	*Effect size (95% CI)*
CTRL	Hipp	L	Encoding	Lure	300	0.81	0.418	0.35 (-0.50, 1.18)
TRAIN	Hipp	L	Encoding	Lure	300	-2.33	0.021	-0.99 (-1.87, -0.09)
CTRL	Hipp	R	Encoding	Lure	300	-0.27	0.788	-0.11 (-0.95, 0.72)
TRAIN	Hipp	R	Encoding	Lure	300	-1.58	0.116	-0.67 (-1.53, 0.20)
CTRL	Hipp	L	Retrieval	Lure	300	1.99	0.048	0.85 (-0.04, 1.71)
TRAIN	Hipp	L	Retrieval	Lure	300	-1.74	0.083	-0.74 (-1.60, 0.13)
CTRL	Hipp	R	Retrieval	Lure	300	1.46	0.146	0.62 (-0.24, 1.47)
TRAIN	Hipp	R	Retrieval	Lure	300	-0.55	0.583	-0.23 (-1.07, 0.61)
CTRL	Hipp	L	Encoding	Repetition	300	0.63	0.526	0.27 (-0.57, 1.11)
TRAIN	Hipp	L	Encoding	Repetition	300	-1.35	0.177	-0.58 (-1.42, 0.28)
CTRL	Hipp	R	Encoding	Repetition	300	0.61	0.541	0.26 (-0.58, 1.10)
TRAIN	Hipp	R	Encoding	Repetition	300	-0.44	0.659	-0.19 (-1.02, 0.65)
CTRL	Hipp	L	Retrieval	Repetition	300	0.79	0.427	0.34 (-0.51, 1.18)
TRAIN	Hipp	L	Retrieval	Repetition	300	-0.96	0.338	-0.41 (-1.25, 0.44)
CTRL	Hipp	R	Retrieval	Repetition	300	0.16	0.874	0.07 (-0.77, 0.90)
TRAIN	Hipp	R	Retrieval	Repetition	300	0.08	0.933	0.04 (-0.80, 0.87)
CTRL	PH	L	Encoding	Lure	300	0.95	0.341	0.41 (-0.44, 1.25)
TRAIN	PH	L	Encoding	Lure	300	-2.97	0.003	-1.26 (-2.17, -0.33)
CTRL	PH	R	Encoding	Lure	300	0.17	0.866	0.07 (-0.77, 0.91)
TRAIN	PH	R	Encoding	Lure	300	-1.24	0.215	-0.53 (-1.38, 0.33)
CTRL	PH	L	Retrieval	Lure	300	1.72	0.087	0.73 (-0.14, 1.59)
TRAIN	PH	L	Retrieval	Lure	300	-2.37	0.019	-1.01 (-1.89, -0.11)
CTRL	PH	R	Retrieval	Lure	300	1.94	0.053	0.83 (-0.06, 1.69)
TRAIN	PH	R	Retrieval	Lure	300	-1.55	0.122	-0.66 (-1.51, 0.21)
CTRL	PH	L	Encoding	Repetition	300	1.10	0.274	0.47 (-0.39, 1.31)
TRAIN	PH	L	Encoding	Repetition	300	-2,25	0.025	-0.96 (-1.84, -0.06)
CTRL	PH	R	Encoding	Repetition	300	1.15	0.250	0.49 (-0.36, 1.33)
TRAIN	PH	R	Encoding	Repetition	300	-0.99	0.321	-0.42 (-1.26, 0.43)
CTRL	PH	L	Retrieval	Repetition	300	1.46	0.146	0.62 (-0.24, 1.47)
TRAIN	PH	L	Retrieval	Repetition	300	-2.01	0.045	-0.86 (-1.73, 0.03)
CTRL	PH	R	Retrieval	Repetition	300	0.65	0.519	0.28 (-0.57, 1.11)
TRAIN	PH	R	Retrieval	Repetition	300	-0.89	0.376	-0.38 (-1.22, 0.47)

*CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. Hipp, hippocampus; PH, parahippocampal gyrus, R, right, L, left, *df*, degrees of freedom, *P*, p-value; Effect size is Cohen's *d* and the corresponding 95% confidence interval (95% CI).

Table S7. Mixed-models of ROI analysis assessing the effects of *Time*, *Group, Laterality, Condition, Stimulus type* and their interaction in the parahippocampal gyrus.*

Effect	***df1***	***df2***	***F***	***P***
Time	1	300	1.65	0.200
Group	1	20	0.04	0.841
Laterality	1	300	1.41	0.236
Condition	1	300	0.22	0.643
Stimulus Type	1	300	0.28	0.597
Time x Group	1	300	34.23	< 0.001
Time x Laterality	1	300	0.81	0.368
Group x Laterality	1	300	0.03	0.852
Time x Condition	1	300	0.57	0.450
Group x Condition	1	300	0.01	0.903
Laterality x Condition	1	300	0.00	0.977
Time x Stimulus Type	1	300	0.15	0.698
Group x Stimulus Type	1	300	1.53	0.217
Laterality x Stimulus Type	1	300	0.00	0.983
Condition x Stimulus Type	1	300	0.02	0.875
Time x Group x Laterality	1	300	2.43	0.120
Time x Group x Condition	1	300	0.19	0.661
Time x Laterality x Condition	1	300	0.05	0.823
Group x Laterality x Condition	1	300	0.03	0.853
Time x Group x Stimulus Type	1	300	0.36	0.548
Time x Laterality x Stimulus Type	1	300	0.01	0.930
Group x Laterality x Stimulus Type	1	300	0.03	0.851
Time x Condition x Stimulus Type	1	300	0.43	0.513
Group x Condition x Stimulus Type	1	300	0.06	0.806
Laterality x Condition x Stimulus Type	1	300	0.02	0.888
Time x Group x Laterality x Condition	1	300	0.09	0.769
Time x Group x Laterality x Stimulus Type	1	300	0.00	0.991
Time x Group x Condition x Stimulus Type	1	300	0.47	0.495
Time x Laterality x Condition x Stimulus Type	1	300	0.08	0.783
Group x Laterality x Condition x Stimulus Type	1	300	0.37	0.545
Time x Group x Laterality x Condition x Stimulus Type	1	300	0.44	0.507

*Mixed-models were performed using *Time* (BDC-4, HDT58), *Group* (TRAIN, CTRL), *Laterality* (Left, Right), *Condition* (Encoding, Retrieval), and *Stimulus Type* (Lure, Repetition) as fixed factors and *Subject* as a random factor. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom; *F*, F-statistics, *P*, p-value.

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Table S8. Contrasts of ROI analysis comparing the BOLD signal change from BDC-4 to HDT58 between groups for bilateral hippocampus and parahippocampal gyrus.*

Region	Laterality	Condition	Stimulus Type	*df*	*t-ratio*	$P_{FDR\text{-}corr.}$	Effect size (95% CI)
Hipp	L	Encoding	Lure	300	-2.22	0.054	-0.95 (-2.07, 0.19)
Hipp	R	Encoding	Lure	300	-0.02	0.609	-0.39
Hipp	L	Retrieval	Lure	300	-2.64	0.035	-1.12 (-2.27, 0.04)
Hipp	R	Retrieval	Lure	300	-1.42	0.609	-0.61
Hipp	L	Encoding	Repetition	300	-1.41	0.215	-0.60 (-1.68, 0.50)
Hipp	R	Encoding	Repetition	300	-0.74	0.609	-0.32
Hipp	L	Retrieval	Repetition	300	-1.24	0.216	-0.53 (-1.61, 0.56)
Hipp	R	Retrieval	Repetition	300	-0.05	0.958	-0.02
PH	L	Encoding	Lure	300	-2.77	0.012	-1.18 (-2.08, -0.26)
PH	R	Encoding	Lure	300	-1.00	0.319	-0.43
PH	L	Retrieval	Lure	300	-2.89	0.012	-1.23 (-2.14, -0.30)
PH	R	Retrieval	Lure	300	-2.47	0.056	-1.05
PH	L	Encoding	Repetition	300	-2.37	0.019	-1.01 (-1.89, -0.11)
PH	R	Encoding	Repetition	300	-1.52	0.260	-0.65
PH	L	Retrieval	Repetition	300	-2.46	0.018	-1.05 (-1.93, -0.14)
PH	R	Retrieval	Repetition	300	-1.08	0.319	-0.46

*Data show effects of the interaction between *Group* (TRAIN, CTRL) and *Time* (HDT58, BDC-4) by *Laterality* (Left, Right) crossed with *Condition* (Encoding, Retrieval) and *Stimulus Type* (Lure, Repetition). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. Hipp, hippocampus; PH, parahippocampal gyrus, R, right, L, left, *df*, degrees of freedom, $P_{FDR\text{-}corr.}$, p-value corrected for multiple comparisons using the Benjamini and Hochberg (BH) false discovery rate procedure; Effect size is Cohen's *d*. 95% CI, 95% confidence interval corrected for multiple comparisons using the false coverage-statement rate (FCR). Note that FCR-adjusted CIs were derived after BH-selection and can only be computed for families containing significant p-values.

Table S9. Contrasts of whole-brain analyses indicating the BOLD signal change from BDC-4 to HDT58 during pattern separation by *Group*.*

	Region	***Direction of effect***	***x y z***	***k***	***Peak Z***	$P_{FWE\text{-}corr.}$
TRAIN	R SPL	Increase	-32 -58 42	25	3.41	0.897
	R OCP	Increase	16 -94 4	135	4.18	0.042
	R MTG	Increase	54 -12 -18	25	3.86	0.897
CTRL	Frontal Pole	Increase	38 36 6	31	3.86	0.815
	R FuG	Increase	28 -56 -18	74	3.83	0.257
	L ITG	Increase	-46 -50 -14	32	3.71	0.800

*Contrasts refer to comparison between BDC-4 (*Lure CR = Foils > Hits*) and HDT58 (*Lure CR = Foils > Hits*). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. R, right; L, left; SPL, superior parietal lobe; OCP, occipital pole; MTG, middle temporal gyrus; FuG, fusiform gyrus; ITG, inferior temporal gyrus; *x, y, z*, MNI coordinates of peak voxel; *k*, cluster size; $P_{FWE\text{-}corr.}$, p-value family-wise error corrected.

Table S10. Contrasts indicating the BOLD signal change from BDC-4 to HDT58 during pattern completion.*

	Region	***Direction of effect***	***x y z***	***k***	***Peak Z***	$P_{FWE\text{-}corr.}$
TRAIN	R SPL	Increase	-32 -58 42	25	3.41	0.897
	R OCP	Increase	16 -94 4	135	4.18	0.042
CTRL	L PrG	Decrease	-38 -16 54	264	4.77	0.002
	R MTG	Decrease	50 -18 -16	29	4.52	0.855
	R PrG	Decrease	30 -6 38	231	4.50	0.005
	R Insular Cortex	Decrease	36 -2 -10	53	4.18	0.512
	L Insular Cortex	Decrease	-34 -10 14	30	4.13	0.841
	L PrG	Decrease	-24 -12 70	37	3.72	0.742
	R PT	Decrease	58 -20 8	24	3.65	0.913
	L PoG	Decrease	-34 -34 62	47	3.64	0.595
Group* x *Time	R PrG		30 -4 42	28	4.12	0.838

* Contrasts refer to comparison between BDC-4 (*Foils* > *Hits* = *Lure CR*) and HDT58 (*Foils* > *Hits* = *Lure CR*). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. L, left; R, right; STG, superior temporal gyrus; OFuG, occipital fusiform gyrus; PrG, precentral gyrus; MTG, middle temporal gyrus; PT, planum temporale; PoG, postcentral gyrus; *x, y, z*, MNI coordinates of peak voxel; *k*, cluster size; $P_{FWE\text{-}corr.}$, p-value family-wise error corrected.

2 Supplementary Figure

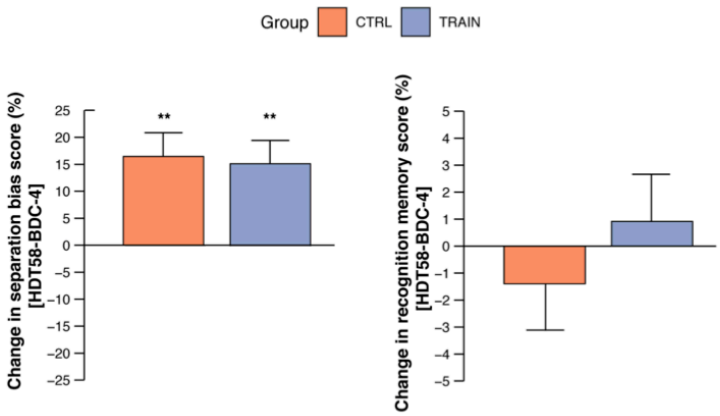

Figure S1. Changes in separation bias and recognition memory score between HDT58 and BDC-4. Data are marginal means and standard errors. CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. ** $P < 0.01$ compared to BDC-4.

Research Paper II

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4	Science Advances	21,901	12.804	0.110010
5	Nature Communications	243,793	11.878	1.103290
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7	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA	661,118	9.580	1.022190
8	Science Bulletin	3,569	6.277	0.009840
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11	Journal of Advanced Research	2,691	5.045	0.004780
12	Research Synthesis Methods	1,932	5.043	0.005420
13	GigaScience	2,674	4.688	0.012510
14	Annals of the New York Academy of Sciences	46,385	4.295	0.025840
15	Scientific Reports	302,086	4.011	1.061540
16	Journal of the Royal Society Interface	12,933	3.224	0.029190
17	NPJ Microgravity	203	3.111	0.000670
18	PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A-MATHEMATICAL PHYSICAL AND ENGINEERING SCIENCES	19,227	3.093	0.028200

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Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization

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The neurobehavioral risks associated with spaceflight are not well understood. In particular, little attention has been paid on the role of resilience, social processes and emotion regulation during long-duration spaceflight. Bed rest is a well-established spaceflight analogue that combines the adaptations associated with physical inactivity and semi-isolation and confinement. We here investigated the effects of 30 days of 6 degrees head-down tilt bed rest on affective picture processing using event-related potentials (ERP) in healthy men. Compared to a control group, bed rest participants showed significantly decreased P300 and LPP amplitudes to pleasant and unpleasant stimuli, especially in centroparietal regions, after 30 days of bed rest. Source localization revealed a bilateral lower activity in the posterior cingulate gyrus, insula and precuneus in the bed rest group in both ERP time frames for emotional, but not neutral stimuli.

Affective processing and emotion regulation are fundamental to human behaviour. They facilitate decision making, have significant influences on learning and memory and provide the motivation for critical action in the face of environmental incentives. The management of positive and negative emotions also directly relates to individual sociability and social interactions. Any emotional alteration may interfere with cognitive performance, impair mental well-being and lead to various forms of psychopathology, especially in the context of a stressful environment[1]. When living and working in an isolated, confined and hostile environment like deep space for prolonged durations, astronauts are exposed to numerous stressors including social isolation, confinement and weightlessness. Currently, the neurobehavioral risks associated with these stressors are not fully understood. In particular, the role of resilience, social processes and emotion regulation during long-duration spaceflight has received little attention so far. Head-down tilt bedrest (HDT) is a well-established model to simulate physical deconditioning and cephalic fluid shifts during standard space missions on the International Space Station (ISS)[2]. Bed rest also comprises a degree of sensory deprivation, isolation, and confinement[3]. Previous studies suggest that long-duration bed rest increases the risk for mood disorders[4], and impairs emotion recognition processing during a Flanker task[5]. According to the authors' knowledge no study has investigated the effects of long-duration bed rest on the neural correlates of emotional processing. The current study aimed to address this gap by investigating the effects of 30 days of -6 degrees HDT bed rest on cortical emotional modulation using event-related brain potentials from a standardized and well-established paradigm[6]. We hypothesized that long-term bed rest would lead to a cortical inhibition of affective processes as indicated by reduced event-related potentials.

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Group	Stimulus	Valence Rating	Arousal Rating
CTRL	pleasant	7.6 (0.6)	5.7 (1.2)
	neutral	4.6 (0.9)	2.4 (1.2)
	unpleasant	2.6 (0.5)	5.7 (1.0)
HDBR	pleasant	7.6 (0.9)	5.7 (1.6)
	neutral	5.2 (0.8)	2.2 (1.2)
	unpleasant	2.6 (0.7)	5.7 (2.2)

Table 1. Subjective ratings for pleasant, neutral and unpleasant IAPS pictures in CTRL and HDBR group. Note: Subjective ratings are based on 9-point Likert scales, ranging from very unpleasant/not arousing at all to very pleasant/very arousing. Data are means and standard deviations.

Results

Emotional self-reports. Table 1 illustrates the self-reported evaluations of each picture category for the control (CTRL) group tested before bed rest, and the intervention group tested after 30 days of head-down tilt bed rest (HDBR). The ratings for all three picture categories were consistent with IAPS normative data[6], confirming the validity of the paradigm in the present experimental setup. In both groups, positive pictures were rated as more arousing and got greater scores for valence than neutral ones (Table 1). Additionally, unpleasant slides received a lower scoring than neutral pictures for valence and were evaluated as more arousing (Table 1). This was confirmed by mixed model analyses, showing a significant main effect of stimulus condition on arousal ($F(2,36) = 76.78$, $p < 0.001$) and valence ($F(2,36) = 309.20$, $p < 0.001$).

However, statistical analyses neither revealed a significant stimulus x group interaction, nor a significant group effect for valence ($F(2,36) = 0.05$, $p = 0.948$ and $F(1,18) = 0.02$, $p = 0.879$, respectively) or arousal ($F(2,36) = 0.10$, $p = 0.909$ and $F(1,18) = 0.08$, $p = 0.928$, respectively). Planned contrasts revealed similar ratings for valence and arousal for all picture categories between groups (all $ps > 0.728$).

Electrophysiological data. Figure 1A depicts the grand average ERP waveforms for CTRL and HDBR subjects in frontal and parietal regions, respectively. While neutral pictures elicited similar responses in CTRL and HDBR participants, the ERP waveforms of emotional stimuli were inhibited in the HDBR group compared to the CTRL group. As shown in Table 2, the mixed ANOVA analysis of mean P300 amplitude revealed a significant interaction of group and stimulus in the frontal ($p = 0.002$) and parietal sites ($p = 0.002$). Mean LPP amplitude showed a significant effect of group in frontal ($p = 0.048$) and parietal sites ($p = 0.026$) and a significant effect of stimulus in parietal site ($p < 0.001$). Simple comparisons are shown in Table S1 and Table S2 that can be found in the Supplementary Information.

Planned contrasts (Table S1) confirmed that emotional pictures induced enhanced electrocortical responses in CTRL compared to HDBR participants in both regions and time frames (all $ps < 0.029$) except for the frontal LPP which was not significant between groups for positive pictures ($p = 0.074$). For the neutral stimuli, no differences in LPP and P300 amplitudes between groups were observed (all $ps > 0.314$). The ERP difference topography between emotional and neutral stimuli for both components and both groups is illustrated in Fig. 1B. While CTRL participants showed enhanced P300 and LPP amplitudes for emotional stimuli relative to neutral pictures, there was no visible difference in the HDBR group. A follow-up analysis using pre-planned contrasts (Table S2) revealed that positive and negative stimuli evoked significantly increased P300 components compared to neutral stimuli in the CTRL (all $ps < 0.003$), but not the HDBR group (all $ps > 0.414$). We also observed significant differences between LPP components induced by positive stimuli and neutral stimuli in both regions (all $ps < 0.037$) and a significantly smaller LPP amplitude in the frontal area induced by negative pictures compared to neutral pictures ($p < 0.001$) in CTRL participants only.

eLORETA data. For the averaged LPP evoked by positive pictures, a significantly lower cortical activation for HDBR compared to CTRL participants was found in the right insula (BA 13, $p < 0.05$, Fig. 2). The P300 comparison between CTRL and HDBR group revealed statistically lower cortical activations in the bilateral precuneus and the bilateral cingulate gyrus (BA 31/7, $p < 0.05$, Fig. 2). Moreover, analysis of P300 and LPP showed a decrease in cortical activity at the same locations (BA 31/7; all $ps < 0.05$, Fig. 2) when processing negative pictures, as compared to CTRL group. No significant differences were found comparing CTRL and HDBR group for mean P300 and LPP amplitudes evoked by neutral stimuli (see $F_{critical}$ in Table 3).

Discussion

The present study investigated the effects of 30 days of immobilization on affective picture processing in young healthy men. To evaluate the impact of long-term bed rest on emotional processing we employed a well-established ERP paradigm using standardized affective stimuli. Our main findings include an inhibition of P300 and LPP components for emotional stimuli, but not neutral pictures in HDBR participants when compared to a sex- and age-matched control group. This inhibition was found to be localized in the precuneus, cingulate gyrus, and insula.

The CTRL group exhibited larger P300 and LPP components when viewing pleasant and unpleasant pictures as compared to neutral slides. This result is well in line with previous research investigating affective picture processing in young healthy adults[7,8]. Larger evoked potentials are thought to reflect increased attention towards biologically relevant emotional stimuli[9]. Particularly, the P300 has been hypothesized to be an index of

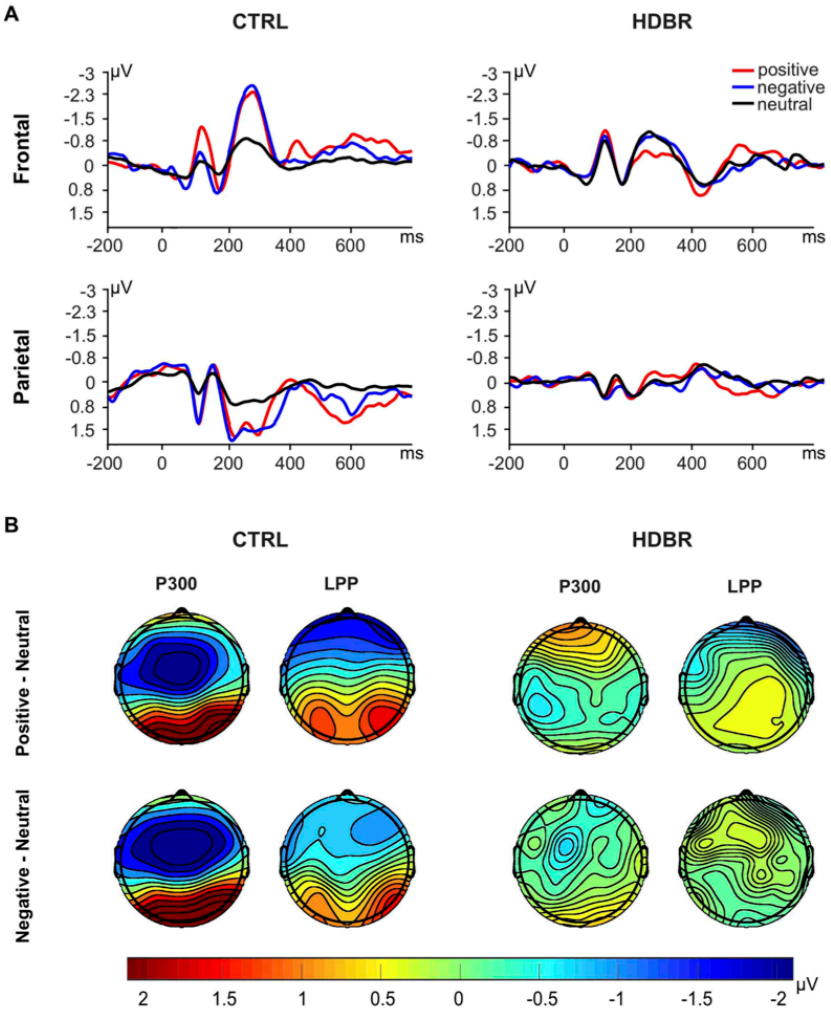

Figure 1. Event-related potential (ERP) results. (**A**) Grand average ERP waveforms at selected electrode clusters (frontal: F3, F4; parietal: P3, P4, Pz) for positive. (n = 25), negative (n = 25) and neutral (n = 25) stimuli in a control group (CTRL, n = 10) and a bed rest group (HDBR, n = 10). (**B**) Topographical maps depicting mean voltage differences between positive and neutral, and between negative and neutral stimuli averaged for the CTRL group and HDBR group for each ERP component (i.e., P300, and LPP).

initial memory storage and attention[10], whereas the LPP is supposed to be a cortical correlate that is associated with encoding and memory processes[11]. Additionally, emotional stimuli are better perceived, encoded, consolidated and retrieved than neutral stimuli[12]. In contrast, we did not observe the expected difference between brain potentials in HDBR participants, immobilized for 30 days in -6 degrees head-down tilt position. We found that long-term immobilization resulted in emotional blunting as evidenced by reduced LPP and P300 amplitudes in response to affective images, i.e., pleasant and unpleasant stimuli elicited a similar flattened response as neutral ones. The emotional blunting indicates dysfunctional modulations in the processing of emotional information.

A source localization revealed a cortical inhibition of distinct brain regions. Specifically, long-term bed rest was found to be associated with a lower activation within the right insula, the bilateral precuneus, and the bilateral posterior cingulate gyrus (PCG) when processing pleasant and unpleasant stimuli. Electrophysiological recordings and neuroimaging have supported key positions of the amygdala, cingulate gyrus and insula in response to emotional stimuli[13]. Moreover, past studies reported a similar role in emotional information processing for

Factor	P300	LLP
Frontal electrode cluster		
Group	$F(1, 18) = 2.84$	$F(1, 18) = 4.50$*
Stimulus	$F(2, 36) = 10.12$***	$F(2, 36) = 1.33$
Group x Stimulus	$F(2, 36) = 7.22$**	$F(2, 36) = 3.03$
Parietal electrode cluster		
Group	$F(1, 18) = 11.16$**	$F(1, 18) = 5.86$*
Stimulus	$F(2, 3\,6) = 3.72$*	$F(2, 36) = 16.48$***
Group x Stimulus	$F(2, 36) = 7.65$**	$F(2, 36) = 2.21$

Table 2. Mixed-model analyses assessing the effects of group (HDBR, CTRL) and stimuli (negative, postive, neutral) on P300 and LLP components. *$p < 0.05$. **$p < 0.01$. ***$p < 0.0001$.

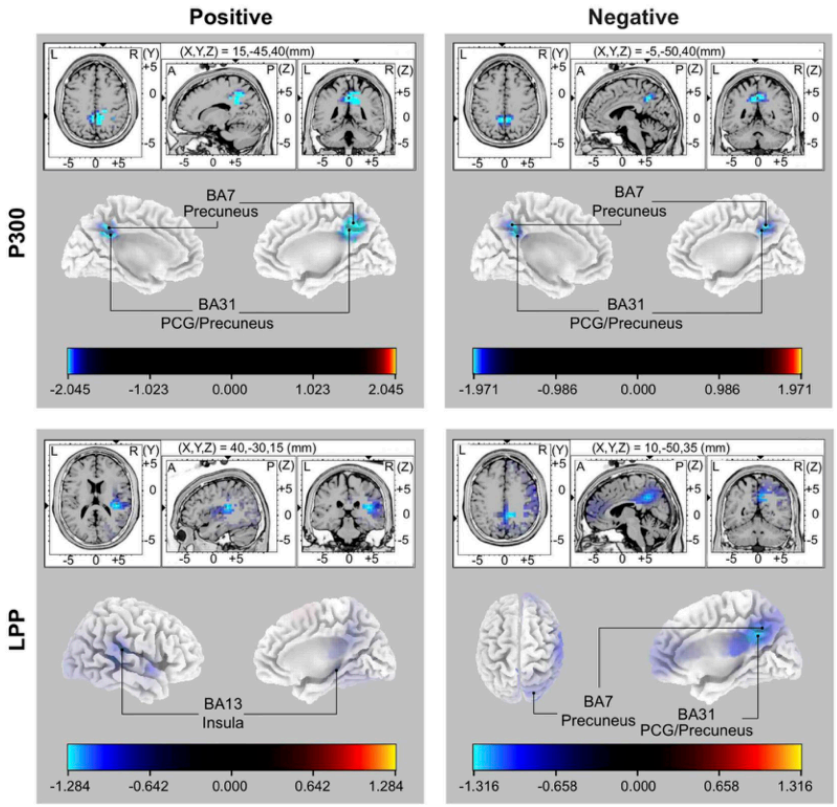

Figure 2. Statistical parametric maps (SPMs) indicating the differences in brain source localization between control (CTRL, n = 10) and head-down-tiltbed rest group (HDBR, n = 10). Data for positive and negative stimuli are shown on the left and right panels, respectively. Results for the P300 and LPP components are provided in the upper and lower panels, respectively. Blue colours indicate decreased activity in the HDBR compared to the CTRL group. The color scale indicates F-values for group differences of brain activity. L left, R right, A anterior, P posterior, PCG posterior cingulate gyrus, BA Brodmann area.

PCG and precuneus due to their structural and functional similarities[14]. There is current evidence, that PCG and precuneus are activated during the evaluation of emotional words[15], the retrieval of emotional memories[16] and the processing of self-relevant affect[17]. The insula, however, plays an important role in pain processing[18]

		Positive	Neutral	Negative
P300	$F_{critical}$ for $p < 0.05$	1.85	1.71	1.78
	$F_{critical}$ for $p < 0.01$	2.05	1.91	1.97
	Statistical Threshold	−2.48	−1.53	−2.02
LPP	$F_{critical}$ for $p < 0.05$	1.24	1.31	1.23
	$F_{critical}$ for $p < 0.01$	1.37	1.51	1.39
	F_{max}	−1.28	−0.88	−1.32

Table 3. Loreta critical thresholds ($F_{critical}$) and maximal *F*-statistics (F_{max}) for ERP components and each stimulus type.

and, additionally, has been shown to be instrumental in the detection, interpretation, and regulation of internal bodily states[19], therefore serving as a critical bridge between affective and cognitive processes. Moreover, precuneus, PCG and insula are reciprocally connected to areas involved in emotional processing such as the anterior cingulate and the orbital frontal cortices, as well as the amygdala[20,21]. Considering these findings, it is reasonable that PCG, precuneus and insula carry emotion-specific information. Interestingly, Zou and colleagues recently showed that 45 days of bed rest altered the resting-state functional architecture of a similar region including the insula and cingulate cortex and hypothesized that these effects might influence the processing of salient information[22]. These data support the vulnerability of these structures to the detrimental neurocognitive effects of prolonged immobilization.

Notably, the self-evaluation of valence and arousal did not differentiate our two groups. The absence of any differences indicates that physiological data may be more objective than behavioural measures as they do not underlie cognitive-social control and are therefore less sensitive to experimental manipulations. Participants possibly tend to respond to self-evaluation in a stereotyped fashion. In line with this, Messerotti and colleagues have shown that acute HDT can suppress cortical emotional responses[23], without affecting behavioural responses. They attribute the electrocortical changes to an altered body position. Recent research performed by the same group has demonstrated that these postural effects on electrocortical activity are immediately observed after changing from sitting to the supine position[24]. To account for postural effects in the present experiment, both groups were tested in the same position, i.e., at -6 degrees HDT, providing sufficient time to account for the cephalic fluid shifts[25]. We therefore assume that the present findings are explained by mechanisms other than acute postural effects.

HDT leads to alterations in brain hemodynamics including an increase in cerebral blood flow (CBF), intracranial pressure, and oxygenated haemoglobin[26], which are hypothesized to trigger cortical inhibition[27]. Additionally, HDT is associated with a cephalic fluid shift leading to increases in thoracic blood volume and hydrostatic pressure, stimulating cardiopulmonary and arterial baroreceptors[2]. These cardiovascular dynamics have been shown to affect cortical activation. Arterial baroreceptors can inhibit cortical activity[28] by decreasing locus coeruleus activity and cortical noradrenaline turnover[29]. Likewise, the blunted responses in HDBR subjects might also be explained by neuroendocrine changes associated with bed rest. Several neurotransmitters are known to be decreased by inactivity including serotonin and norepinephrine[30]. The monoaminergic system which includes norepinephrine and serotonin is well-known for its critical role in controlling human behaviour[31] and in several psychiatric disorders such as depression[32], anxiety[33], and behavioural disturbances among people with dementia[34]. A change in monoamine concentrations associated with long-duration immobilization[35] could therefore also contribute to the changes in visual affective processing observed in the present study. Future studies should therefore also combine behavioural, brain functional, cardiovascular and neuroendocrine measures that will allow to better understand such mechanisms. We also acknowledge that we chose a between-subjects design to exclude any learning effects. Direct between-subject comparisons can be biased by various factors associated with the heterogeneity of the two groups. However, all participants underwent intensive psychological and medical screening for their inclusion in the bed rest study, and they were carefully matched and randomly assigned to one of the two groups. Resting state EEG measured eight days before the intervention, confirmed that EEG spectral power did not differ between the two groups. However, future studies are certainly needed to verify these findings using a within-subjects design in a larger cohort.

Taken together, our data show that head-down tilt bed rest can have adverse neurobehavioral effects associated with negative and positive valence. Impaired affective picture processing following prolonged bed rest was evidenced by a reduction in LPP and P300 in specific brain areas including the insula, precuneus and cingulate gyrus. These results highlight the pervasive effects of physical inactivity that go beyond cardiovascular and musculoskeletal deconditioning. They could have important implications for situations, in which physical activity levels are markedly limited such as during long-duration spaceflight, the aging population, in bed-confinement during hospitalized based care, and people with sedentary lifestyles. Future research needs to elucidate the mechanisms underlying the effects of physical inactivity, examine inter- and intraindividual vulnerabilities relative to emotional regulation, and identify the interaction of physical inactivity and other stressors.

Methods

The present experiment was part of a European Space Agency (ESA) sponsored bed rest study performed at the facilities of the French Institute for Space Medicine and Physiology (MEDES), Toulouse, France in 2017. The project has been registered in the Clinical Trial.gov database under NCT03594799. It comprised 15 days of baseline data collection, 60 days of -6 degrees HDT bed rest and 15 days of recovery. It was conducted following

the Declaration of Helsinki for Medical Research Involving Human Subjects and approved by the Comité de Protection des Personnes (CPP Sud-Ouest Outre-Mer I), the French Health Authorities (Agence Française de Sécurité Sanitaire des Produits de Santé) and the Ethics Committee at Charité–Universitätsmedizin Berlin. All participants were informed about the purpose, experimental procedures, and risks before giving their verbal and written informed consent.

Participants. Data was collected from 20 young healthy male participants (mean age = 34 years, SD = 8; mean height = 176 cm, SD = 4.7; mean weight = 74.0 kg, SD = 7.1; n = 17 right-handed). Handedness was assessed using the Edinburgh Handedness Inventory[36]. Sample sizes were based on previous bed rest studies, suggesting neurobehavioral effects for bed rest[4,5]. We also performed sensitivity analyses for our main outcome, i.e., the comparison of ERP between the bed rest (HDBR) and the control (CTRL) group. For a two-sided independent t-test, a level of significance of 0.05, and a power of 80%, a significant difference corresponding to a Cohen's d of 1.32 should be detectable. This effect is much larger than in a previously reported study using the identical paradigm to assess the acute effects of head-down tilt bed rest[23]. We were therefore confident that the current sample size would be sufficient to reveal a significant between-subjects effect for our primary outcome. All volunteers had no personal history of neurological or psychiatric illness, drug or alcohol abuse, or current medication, and they had a normal or corrected-to-normal vision. The subjects were randomly assigned to one of two groups in a counterbalanced fashion. One of the group served as a control (CTRL: mean age = 34 years, SD = 7; mean height = 176 cm, SD = 3.5; mean weight = 73.1 kg, SD = 5.4) and was tested 8 days prior to bed rest in a -6 degrees HDT position after an adaptational period of 30 minutes of rest. The experimental group (HDBR: mean age: 34 years, SD = 8; mean height = 176 cm, SD = 5.6; mean weight = 74.9 kg, SD = 6.5) was tested after 30 days of (-6 degrees HDT) immobilization. Study cohorts did not differ in age and anthropometric factors (all $ps > 0.740$). Moreover, spectral power analysis of resting state EEG data collected eight days before bed rest revealed no significant difference between groups (data not shown, $p = 0.420$).

Stimuli. Seventy-five standardized stimuli were selected from the IAPS dataset[6] including unpleasant (n = 25, e.g., scenes of violence, threat and injuries), pleasant (n = 25, e.g., sporting events, erotic scenes) and neutral pictures (n = 25, e.g., household objects, landscapes) and presented in a random order. The normative valence ratings (mean (SD)) for each picture category were 7.55 (0.40), 4.99 (0.26), and 3.00 (0.81), and the normative arousal levels (mean (SD)) for each stimulus type were 6.31 (1.10), 2.63 (0.52) and 5.19 (0.61) for positive, neutral and negative images, respectively. The catalogue numbers of pictures from the IAPS dataset used in this study can be found in Supplementary Information.

Procedure. Subjects were positioned in -6 degrees HDT in a dimly lit sound-attenuated room. Testing was performed using a desktop computer (PCGH-Supreme-PC, Alternate), with a 21.5-in monitor (Iiyama ProLite, 1 ms response time, 55–75 Hz refresh rate, luminance 250 cd/m2) installed approximately 60 cm apart from the participant. Before each trial, a central fixation cross appeared for 500 ms. Pictures were displayed on the screen for 2000 ms. After each picture presentation participants were asked to rate the arousal and valence of their emotional perception using two independent 9-point self-assessment Likert scales (SAM) that ranged from very unpleasant/not arousing at all to very pleasant/very arousing[37]. The rating was performed using a computer mouse without any time constraints. The accuracy was emphasized to ensure response reliability and maximal attention from the subjects to their feelings.

EEG recording. The electrocortical activity was continuously recorded and synchronized with the stimuli using an active electrode 32-channel amplifier (actiCHamp, Brain Products GmbH, Germany). Picture presentation and timing were controlled through the use of Presentation software version 18.1 (Neurobehavioral Systems, Inc., USA). Electrodes were attached to an EEG cap (actiCap, Brain Products GmbH, Germany) and placed at positions Fp1, F3, FT9, FC5, FC1, T7, TP9, CP5, CP1, P7, P8, TP10, CP6, CP2, T8, FT10, FC6, FC2, Fp2, F7, F8, F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1 and O2 in accordance with the International 10–20 System. Signals were referenced to Fz. Electrode impedance was checked for each subject before data collection and maintained at less than 5 kΩ. Eye movements and eye blinks were monitored via tin electrooculogram (EOG) electrodes (B18 Multitrodes, EASYCAP GmbH, Germany) placed above and below the left eye as well as at the outer canthi of both eyes. EEG and EOG signals were amplified by a multi-channel bio-signal amplifier and A/D converted at 1000 Hz per channel with 24-bit resolution.

EEG data processing. The data were analysed offline employing EEGLAB 14.0.0[38], a toolbox embedded in Matlab R2015b (The MathWorks, Inc., Natick, Massachusetts, United States). First, data were filtered using a 0.1 to 40 Hz band pass filter. Then, recordings were visually inspected allowing also an interpolation of bad channels. After re-referencing to average reference, EEG data were epoched to the respective stimulus presentation including 200 ms of pre-stimulus baseline and 800 ms of stimulus-dependent data. EOG artefacts were removed using vertical and horizontal EOG regression channels[39]. Muscle artefacts were removed using a spatial filtering framework with defaults[40]. After baseline removal, ERPlab 6.1.3[41] was used to run an additional automated exclusion procedure, rejecting epochs which exceed a gradient threshold of 50 μV, or a maximum and minimum amplitude of ± 100 μV. A total of 2.1% of the trials were excluded in the CTRL group, while 1.1% of the trials had to be excluded for the HDBR group. Average ERPs were computed separately for each subject and each condition. Further, the waveforms were transformed into topographic maps of the ERP potential distributions. The LPP was measured as the average voltage of 400 to 700 ms following picture onset. The P300 was measured as the average voltage of 280 to 350 ms after stimulus presentation. Mean P300 and LPP amplitude was averaged for F3 and F4 as well as P3, P4 and Pz to assess frontal and parietal activity, respectively. A digital 12 Hz low-pass filter was applied offline for plotting grand-averaged waveforms while electrophysiological activity using original filter settings was used for all statistical analyses.

Time-dependent cortical localization of EEG activity. Source analysis was performed by exact low-resolution brain electromagnetic tomography (eLORETA, http://www.uzh.ch/keyinst/loreta.htm), enabling the spatial identification of the cortical activity. The eLORETA software employs a discrete, three-dimensional distributed, linear, weighted minimum norm inverse solution method. The particular weights used in eLORETA allow for an exact localization to test point sources and provide better localization of highly correlated point sources with low signal to noise ratio data[42]. Three-dimensional solution space is restricted to cortical gray matter, as determined by the probabilistic Talairach Atlas. The brain compartment includes 6239 voxels with 5 mm spatial resolution. Anatomical labels, i.e., Brodmann areas (BA) are reported using MNI space, with correction to Talairach space.

In order to receive the 3D cortical distribution of the electrical neuronal generators, the electrode positions were applied to a probabilistic anatomical template of the Talairach Atlas. The Talairach coordinates were used to compute the eLORETA transformation matrix. The eLORETA files were obtained, using the transformation matrix and the ERP data of each subject for each stimuli type. The transformed eLORETA files, containing the corresponding 3D cortical distribution of the electrical neuronal generators, were used for further statistical analysis.

Statistical analysis. *Differences in the temporal dynamics of ERP maps.* Descriptive statistics are reported as means and standard deviations (SD). To test for differences in self-reported evaluations of emotional valence and arousal we performed two-factorial mixed linear models. Subjects were entered as random factors and group (CTRL, HDBR) and stimulus type (positive, neutral, negative) were included as fixed factors, respectively. Further, a mixed-model design was employed to compare the ERP components between groups (CTRL, HDBR) and stimulus type (positive, negative, neutral). Separate mixed model ANOVAs were run for each combination of region (frontal, parietal) and ERP component (P300, LPP). Stimulus type and group were entered as fixed factors and subjects as random effects. Simple comparisons for each condition were performed using pre-planned contrasts with corrections for multiple comparisons[43]. Effect sizes were reported as Cohen's *d*. Confidence intervals of effect sizes were bootstrapped using 2000 resamples[44]. All statistical analyses were carried out using the software package R version 3.5.1[45]. Mixed models were run using the packages lme4[46] and lmerTest2[47]. The level of significance was set at $\alpha = 0.05$ (two-sided) for all testing.

Time-dependent localization of significant differences in temporal dynamics. Independent sampled F-tests were used to test for differences in estimated cortical current density between CTRL and HDBR in all emotional conditions and both time frames. Statistical significance was assessed using a non-parametric randomization test with 5000 randomizations that determined the critical probability threshold ($F_{critical}$) with corrections for multiple testing[48]. As a result, each voxel was assigned a F-value. Voxel-by-voxel F-values are displayed as statistical parametric maps (SPMs).

Data availability

The datasets that support the findings of the current study are available from the corresponding author on reasonable request.

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Author contributions

A.S. conceived, designed, planned, and supervised the experiment. K.B. drafted the manuscript and processed the data. A.W. performed data collections with support from K.B. A.W., M.A.M. D.F.D. and H.C.G. provided critical feedback and contributed to the interpretation of the results. All authors discussed the results and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization

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Supplementary Information

Supplementary Study Materials

Study stimuli

The following pictures from the International Affective Picture System, listed by catalog number, were used in the current study: negative - 1114, 1205, 1110, 3064, 3140, 3168, 3250, 3261, 9140, 9265, 9301, 9419, 9420, 9433, 9520, 9592, 9800, 9925, 9926, 9561, 9341, 9342, 9340, 3230, 9584; neutral - 2235, 7057, 5390, 5731, 5740, 7002, 7004, 7006, 7009, 7010, 7056, 7025, 7041, 7050, 7052, 7053, 7055, 7059, 7060, 7080, 7090, 7150, 7175, 7233, 7235; positive - 1440, 8185, 1460, 5629, 1710, 1722, 1750, 2071, 8260, 2311, 8185, 4002, 4006, 4141, 4142, 4180, 4225, 4232, 4250, 4255, 4652, 4659, 4694, 4695.

Supplemental Results

Supplemental Study Table

Table S1. Contrasts comparing ERPs for negative, neutral and positive stimuli between control (CTRL) and bed rest (HDBR) groups.*

ERP	Stimulus	*df*	*t*	*p*	*d* [95%CI]
P300 frontal	negative	30	2.52	0.017	-1.15 [-1.98, -0.31]
	neutral	30	-0.45	0.659	0.30 [-0.65, 1.37]
	positive	30	2.29	0.029	-0.81[-1.70, -0.06]
P300 parietal	negative	30	-3.81	<0.001	1.56 [0.69, 2.22]
	neutral	30	-1.03	0.314	0.78 [-0.11, 1.63]
	positive	30	-3.93	<0.001	1.45 [0.75, 2.04]
LPP frontal	negative	39	2.90	0.006	-1.25 [-1.09, 0.73]
	neutral	39	0.33	0.745	-0.25 [-0.65, 1.37]
	positive	39	1.83	0.074	-0.65 [-1.69, 0.35]
LPP parietal	negative	44	-2.37	0.022	0.98 [0.13, 1.96]
	neutral	44	-0.44	0.659	0.21 [-0.83, 1.01]
	positive	44	-2.65	0.011	1.21 [-0.10, 2.19]

*_df_, degrees of freedom; _d_, effect size (Cohen's _d_) and 95% confidence intervals (CI). CIs are bootstrapped using 2000 resamples.

Supplemental Study Table

Table S2. Contrasts comparing ERPs between stimuli conditions (negative vs. neutral and positive vs. neutral) in control (CTRL) and bed rest (HDBR) groups.*

ERP	Group	Stimulus	*df*	*t*	*p*	*d* [95% CI]
P300 frontal	CTRL	negative - neutral	36	-5.52	<0.001	-1.76 [-2.76, -1.02]
		positive - neutral	36	3.17	0.003	-0.71 [-1.39, -0.06]
	HDBR	negative - neutral	36	-0.69	0.497	-0.21 [-0.86, 0.54]
		positive - neutral	36	1.29	0.414	0.48 [-0.35, 1.26]
P300 parietal	CTRL	negative - neutral	36	4.25	<0.001	1.19 [0.58, 1.75]
		positive - neutral	36	3.62	<0.001	0.82 [0.19, 1.43]
	HDBR	negative - neutral	36	-0.43	0.669	-0.12 [-0.82, 0.56]
		positive - neutral	36	-1.27	0.424	-0.48 [-1.17, 0.18]
LPP frontal	CTRL	negative - neutral	36	-2.36	0.037	-0.88 [-1.53, -0.19]
		positive - neutral	36	-2.17	0.037	-0.65 [-1.48, -0.06]
	HDBR	negative - neutral	36	1.11	0.552	0.45 [-0.26, 1.36]
		positive - neutral	36	-0.14	0.891	-0.04 [-0.74, 0.69]
LPP parietal	CTRL	negative - neutral	36	1.22	0.230	0.42 [-0.26, 1.23]
		positive - neutral	36	4.89	<0.001	1.88 [0.62, 3.98]
	HDBR	negative - neutral	36	-1.16	0.252	-0.30 [0.62, 3.98]
		positive - neutral	36	2.17	0.074	0.57 [-0.02, 1.15]

*_df_, degrees of freedom; _d_, effect size (Cohen's _d_) and 95% confidence intervals (CI).
CIs are bootstrapped using 2000 resamples.

Research Paper III

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34	INTERNATIONAL JOURNAL OF PSYCHOPHYSIOLOGY	8,822	2.631	0.009440
35	EUROPEAN JOURNAL OF APPLIED PHYSIOLOGY	16,418	2.580	0.012130

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Impaired Attentional Processing During Parabolic Flight

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Previous studies suggest that altered gravity levels during parabolic flight maneuvers affect spatial updating. Little is known about the impact of the experimental setting and psychological stressors associated with parabolic flight experiments on attentional processes. To address this gap, we investigated the level of alertness, selective and sustained attention in 1 and 0 g using a Go/No-Go Continuous Performance Task. We also identified several parameters associated with the experimental set-up of a parabolic flight that could be expected to affect attentional processing. These included the use of scopolamine, sleep quality prior to the flight day, participant's stress level as well as mood and anxiety state before and after the parabolic flight. We observed a deterioration in attentional processing prior to the first parabola that was further aggravated in weightlessness and returned to baseline after the last parabola. *Reaction Time*, *Hit* and *False Alarm Rate* were moderately correlated with self-reported anxiety state, but not cortisol levels or emotional states. The use of scopolamine had minor effects on *Reaction Time*. Our results confirm previous studies reporting impairments of cognitive performance in 0 g, and highlight important aspects that should be considered for the design of behavioral research experiments in future parabolic flight campaigns.

Keywords: microgravity, attention, scopolamine, anxiety, human, adverse effects

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INTRODUCTION

With the resurgence of interest in space exploration and human settlement in space, researchers are seeking to better understand the effects of gravity on the human body and to ensure safe and successful space exploration. The central nervous system has been brought into focus of these investigations, and there is a growing interest in better understanding the effects of spaceflight on brain and behavior (Roberts et al., 2020). Recent studies have reported structural brain changes

using magnetic resonance imaging (MRI) after prolonged space flight (Demertzi et al., 2016; Roberts et al., 2017; Van Ombergen et al., 2018, 2019) and alterations in functional connectivity after exposure to different gravity conditions, i.e., short periods of hyper- and hypogravity during parabolic flights (Van Ombergen et al., 2017). Evidence from spaceflight research has also reported that weightlessness led to altered spatial cognition abilities (Paloski et al., 2008; Cheron et al., 2014), and impaired sensory-motor integration and control (Casellato et al., 2012; Hallgren et al., 2016; Reschke et al., 2017). Likewise, Stahn et al. (2020) and others have shown that spatial cognition is significantly impaired during altered gravity conditions (Grabherr et al., 2007; Grabherr and Mast, 2010; Clément et al., 2016). In contrast to these studies, Wollseiffen and colleagues reported faster reaction times for a complex mental arithmetic task (Wollseiffen et al., 2016) as well as in combination with an oddball task paradigm (Wollseiffen et al., 2019) in microgravity during parabolic flight. To fully understand the effects of altered gravity conditions on neurobehavioral performance, it is important to disentangle the effects related to microgravity from potential confounders associated with parabolic flight maneuvers *per se*. Factors such as an increased stress and anxietly level, especially for first-time flyers, and poor sleep prior to the flight day may impact behavioral measures. Further, participants may also experience severe motion sickness that is typically attenuated by an antiemetic drug administered before the flight. A lack of sleep and poor sleep quality, antiemetic drugs, mood, and stress, each of which can confound cognitive and motor performance (Wesnes and Warburton, 1983; Lim and Dinges, 2008; Bestaven et al., 2016), functions that are known to be dependent on the level of attention and selective attention abilities (Carrasco, 2018; Ruff and Cohen, 2019; Song, 2019).

Here, we investigated the level of alertness, selective and sustained attention using a Go/No-Go Continuous Performance Task (CPT) in male first-time flyers before, during, and after parabolic flight exposure. As a secondary outcome, we assessed parameters associated with the experimental set-up of the parabolic flight campaign that we expected to affect attentional processing, i.e., the use of an antiemetic drug, stress level, participant's mood and anxiety state as well as their sleep quality prior to the flight. All paradigms and questionnaires have been validated in previous behavioral research (Rosvold et al., 1956; McNair et al., 1981; Spielberger et al., 1983; Drummond et al., 2005; Golding et al., 2017) and were adapted when necessary to the parabolic flight constraints. We hypothesized that the level of alertness and attention is impaired by the weightlessness as well as by the experimental set-up of the parabolic flight campaign itself, and that both factors would influence cognitive performance.

MATERIALS AND METHODS

Participants

Twelve men (mean age: 48.75 years ± 8.7, range: 34–55 years) participated in the study. All participants were naïve to the experience of microgravity, non-smokers, free of any cardiovascular, vestibular, psychiatric, and neurological disorders, had a normal or corrected-to-normal vision, and passed a Class 3 Aviation medical exam. Approximately 75–90 min prior to the take-off, all participants received 0.175 mg of scopolamine that was injected subcutaneously by the campaign's flight physician.

Study Design

Data were collected on board of an Airbus 310 Zero-G during the 131st parabolic flight campaign operated by Novespace[1]. The campaign took place in October 2017 in Bordeaux Merignac, France, and was sponsored by the Centre national d'étude spatiales (CNES). The campaign was composed of a familiarization day that was used to collect baseline data, followed by three days of parabolic flights. Each flight started in the morning at about 9h30 am, and was finished at about 1 pm. The flight consisted of 31 parabolas, each starting and ending with a hypergravity phase of 1.8 g of approximately 20 s, and a microgravity[2] phase of approximately 22 s in-between. Averaged values of the hypogravity periods were 0.0095 g, 0.0036 g, and 0.004 g for x-, y-, and z-axis, respectively. The averaged g-levels during each parabola are provided in **Supplementary Table 1**. All participants were familiarized with the test protocol on the first day of the campaign and participated in one parabolic flight, i.e., on one of the three consecutive flight days. On the flight day, participants were offered an antiemetic drug (scopolamine) as a voluntary routine option because scopolamine has been shown to decrease the risk of motion sickness compared with no medication intake during parabolic flights (Golding et al., 2017). All participants volunteered to received scopolamine in the morning prior to the flight. The experiment was approved by the Comité de Protection des Personnes Nord Ouest III, Caen, France (HYPOCAMPUS 2015-A02014-45) and conformed to all standards of human research set out in the declaration of Helsinki. All participants were informed about the purpose, experimental procedures, and the risks before giving their verbal and written informed consent. Four participants were tested per flight day. **Figure 1** displays a schematic overview of the study design.

Inflight-Testing

Go/No-Go Paradigm. We administered a Go/No-Go Continuous Performance Task (CPT) for recording selective and sustained

[1]https://www.airzerog.com

[2]During this phase, the aircraft flies a parabolic arc producing a freefall wherein the acceleration of the aircraft cancels the acceleration due to gravity along the aircraft vertical z-axis. With respect to an external fixed reference frame, the aircraft and all passengers and objects inside of the plane fall together with an acceleration of 9.81 m/s^2. There is no reaction force on the passengers by the aircraft and a net level of 0 g is achieved generating the perception of microgravity or zero-gravity (Karmali and Shelhamer, 2008). Accordingly, the terms micro-, hypo-, and zero-gravity are technically incorrect because gravity is still 1 g during the entire flight maneuver. However, in the manuscript we follow the commonly used terminology in Space Life Science and use the terms interchangeably to describe a net level of 0 g during the freefall.

attention as well as impulsive behavior. The test has been introduced by Rosvold et al. (1956) and, since then, has been implemented in different forms in the research on attentional processes. Single letters were presented in random order on the computer screen. Every 920 ms a letter appeared and was displayed for the same duration. During the 0 g condition, participants were asked to react to a specific target letter (X) by pressing the space bar with their index as soon as the target appeared and withhold responses to all other stimuli. Targets appeared in 30% of the trials, i.e., 7 targets of 22 trials per parabola. The task was programmed and applied using the VRmaze software (Machado et al., 2019), adapted and shortened according to the requirements and time constraints of the parabolic flight maneuver, and presented on a 15-inch laptop (ZBook 15 G5 Mobile Workstation, Hewlett Packard). Cognitive data were collected at the following points in time: (1) once during familiarization session on the day of arrival at Novespace; (2) on the flight day before and after scopolamine injection; (3) inflight at 1 g before experiencing the first parabola, during 0 g, and inflight at 1 g after the last parabola; and (4) post-flight (**Figure 1**). Data collected at the time points before and after scopolamine injection were used to evaluate the effect of antiemetics (see section "Scopolamine" for further information). For the analysis of the data collected inflight during 1 and 0 g, parameters obtained post-medication were used as a baseline to avoid any further bias. Data were acquired in a seated position during 1 g onboard of the plane and in a controlled free-floating position during 0 g condition (**Figure 2A**). A fabric-covered rack was used to minimize visual distractions throughout all conditions. Participants performed the CPT either from parabola 11 to 15 or from parabola 26 to 30 (**Figure 2**). During the remaining parabolas the participants performed a virtual navigation task, which will be reported elsewhere. Performance was quantified by (1) *Reaction Time* (RT) for target trials in milliseconds (ms); (2) *Hit Rate* (correct detection of the target letter) in percentage to determine attentional capacity; and (3) *False Alarm Rate* (Reaction to non-targets) in percentage as an indicator of

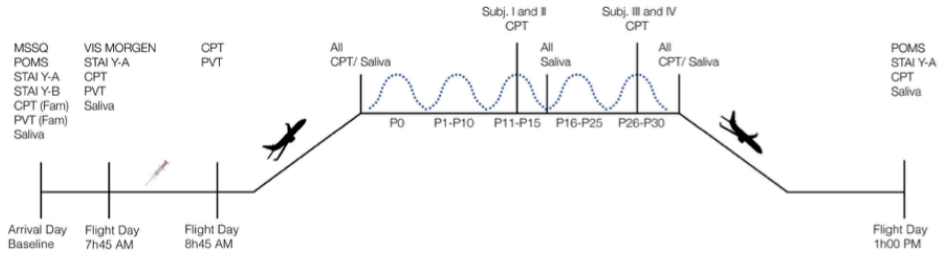

FIGURE 1 | Schematic overview of the study design. Four participants were tested on each flight day. MSSQ, Motion Sickness Susceptibility Questionnaire; POMS, Profile of Mood States; STAI Y-A/Y-B, State-Trait Anxiety Inventory Form Y Part A/B; CPT, Continuous Performance Task; PVT, Psychomotor Vigilance Task, Fam, familiarization session; VIS Morgen, sleep questionnaire; P, Parabola; syringe represents subcutaneous scopolamine injection.

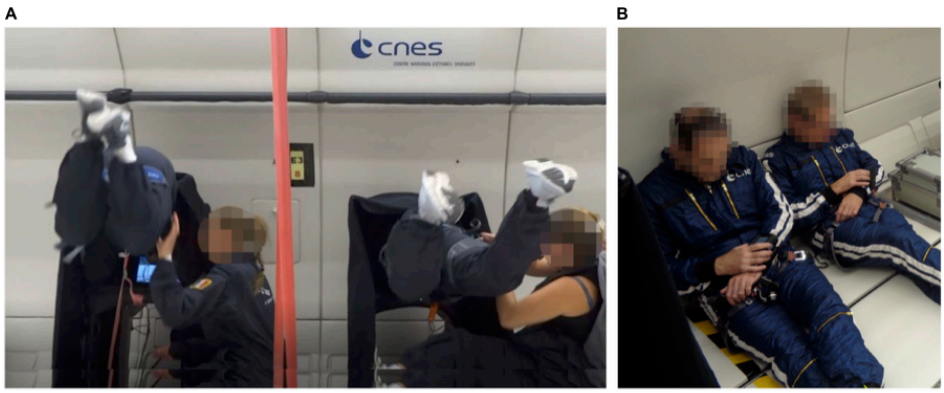

FIGURE 2 | Experimental set-up. **(A)** Participants performing the Continuous Performance Task (CPT) in a controlled free-floating position. **(B)** Participants performing the psychomotor vigilance test (PVT) using a diving computer that was attached to participants' wrist. During both tasks, two participants were tested at the same time.

impulsivity. Additionally, we also computed *d-prime* (d') as an indicator of sensitivity. The *Omission Error Rate* in percent as an indicator of distraction is reported descriptively, because the inferential statistical characteristics are identical to those of the *Hit Rate*.

Testing of Factors Associated With the Experimental Set-Up of the Parabolic Flight Campaign

Motion Sickness

To evaluate participant's susceptibility to motion sickness, we administered the short form of the Motion Sickness Susceptibility Questionnaire (MSSQ) on the day of arrival. The questionnaire has been validated and previously used during parabolic flight campaigns (Golding et al., 2017). By assessing previous experience of motion sickness symptoms and nausea in different transport modes during child- and adulthood, a raw score between 0 and 54 is calculated and a percentile conversion is given (Golding, 2006). Irrespective of the test result, an antiemetic drug was offered to all participants of the experiment to avoid severe motion sickness symptoms.

Scopolamine

Scopolamine is a muscarinic antagonist that is often used for preventing nausea and motion sickness symptoms (Lochner and Thompson, 2016) but also provokes drowsiness and fatigue and has been reported to disrupt performance in tests of sustained attention (Wesnes and Warburton, 1983). During parabolic flight campaigns, scopolamine is offered as a routine to avoid severe motion sickness symptoms that may occur in up to 90% of first-time flyers. The sedative and antiemetic effect of scopolamine occurs approximately 30 min after medication. To identify whether scopolamine impacts participants' attentional processing, we also administered the CPT on the flight day before and 30 min after subcutaneous scopolamine injection. Additionally, we also employed a Psychomotor Vigilance Task (PVT) before and after medication to assess participants' vigilance. The test has been validated by means of functional magnetic resonance imaging (fMRI) (Drummond et al., 2005). A visual stimulus in form of a red dot appeared ten times at random interstimulus intervals throughout a total test duration of 2 min. Participants were asked to press a button as quickly as possible each time the red dot would appear and *Reaction Time* was recorded. The PVT was an adapted version of the test described by Moore et al. (2017) and was administered using a modified diving computer (MARES Icon®) that was attached to participants' wrist (Balestra et al., 2018). The test was performed in a seated position in an open space area in the aircraft (**Figure 2B**).

Stress

Salivary cortisol was collected using the Salivette® (Sarstedt, Nümbrecht, Germany) cotton swab system to assess participant's stress response. All participants were familiarized with the correct sample collection, i.e., avoid eating, drinking, and brushing teeth at least 30 min prior to sample collection, and chew on the cotton swab for 60 s. Saliva samples were collected on the day of arrival before noon, in the morning of the flight day after wake-up, before experiencing the first parabola (P0), after the 15th and 30th parabola (P15, P30), and post-flight. Samples were subsequently frozen and stored at a temperature of −25°C. Cortisol concentrations were then quantified by an electrochemiluminescence immunoassay (ECLIA, Roche, Mannheim, Germany) on a *cobas e411* analyzer at the University Hospital of Caen, Normandy, France. To verify whether cortisol levels were not affected by sleep quality, we also calculated the change in salivary cortisol from P0 to P30 and correlated this change with self-reported sleep quality and total sleep duration (see also section "Sleep Quality" for sleep assessment).

Anxiety and Mood State

Anxiety level was assessed using the French version (Form Y) of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). Trait anxiety as a personal characteristic was determined only once on the day of arrival. State anxiety was assessed three times: on the day of arrival at Novespace, before take-off, and after landing. To evaluate changes in mood states, a validated French version of the Profile of Mood States Questionnaire (POMS) (Cayrou et al., 2003) was administered on the day of arrival and after the parabolic flight.

Sleep Quality

To evaluate participants' sleep quality and sleep duration of the preceding night of the parabolic flight, the VIS-Morgen Questionnaire was administered. The questionnaire was originally introduced by the *Centre du Sommeil et de la Vigilance, Hôpitaux universitaires, Paris Centre* and assesses sleep quality and participant's energy upon awakening on a visual analog scale between 0 and 10 (Dubois et al., 2013). It also records the number of perceived wake-ups and sleep duration. The questionnaire was administered on the flight day before boarding the plane.

Association Between CPT Performance and Factors Associated With the Experimental Set-Up of a Parabolic Flight Campaign

To identify the relationships between attentional processes and emotional state, anxiety, and stress, we performed an exploratory analysis and correlated CPT data (*Reaction Time, Hit Rate, False Alarm Rate*) collected during 1 g inflight (before P0 and after P30) with salivary cortisol (before P0 and after P30), and with mood states (POMS questionnaire administered on-ground immediately before and after flight). We also correlated CPT data (on the day of arrival, before P0, and after P30) with state anxiety score (on the day of arrival, on-ground immediately before and after parabolic flight exposure) at all three time points.

Statistical Analysis

Descriptive statistics are presented as marginal means and standard errors of the mean (*SE*) unless stated otherwise.

Differences between points in time were assessed using a linear mixed model with *Time* as a fixed factor, and *Subject* as a random factor (random intercept only). Pre-planned contrasts were computed for simple comparisons between points in time with a sequential Holm – Bonferroni correction for multiple comparisons (Holm, 1979). Effect sizes are reported as Cohen's *d* and 95% confidence intervals (CI). The relationships between CPT variables and mood states, anxiety, and stress were determined using repeated measures correlation. The level of significance was set at $\alpha = 0.05$ (two-sided) for all tests. Estimated marginal means were calculated using emmeans package (Lenth, 2016), effect size and confidence intervals were computed using psych package, the sensitivity index d' was calculated using psycho package, version 0.5.0, correlation analysis was performed using rmcorr package (Bakdash and Marusich, 2017), and figures were created using ggplot2 (Wickham, 2016). All statistical analyses and graphical illustrations were carried out using the software package R (R Core Team, 2018).

RESULTS

Inflight Testing

Compared to pre-flight, a deterioration in task performance was observed in 1 g before experiencing the first parabola (1 g before P0) and during 0 g, but not in 1 g after the last parabola (1 g after P30) and post-flight. The decline in task performance was characterized by a significantly longer *Reaction Time* [$t_{43.2} = 2.98$, $P = 0.019$, $d = 0.86$ (0.18, 1.52) and $t_{43.2} = 2.58$, $P = 0.040$, $d = 0.74$ (0.09, 1.37)], by a lower *Hit Rate* [$t_{43.1} = -2.93$, $P = 0.016$, $d = -0.85$ (−1.50, −0.17) and $t_{43.1} = -5.11$, $P < 0.001$, $d = -1.47$ (−2.29, − 0.63)], and by a higher rate of *False Alarms* [$t_{42.9} = 3.66$, $P = 0.002$, $d = 1.06$ (0.33, 1.76) and $t_{42.9} = 4.71$, $P < 0.001$, $d = 1.36$ (0.55, 2.14)] during 1 g before P0 and during 0 g, respectively (**Figures 3A–C**). At all points in time, d' exceeded a value of two, suggesting that participants were generally able to discriminate the signal over noise. In line with the performance decline, d' also decreased significantly in 1 g before P0 [$t_{42.9} = -4.31$, $P < 0.001$, $d = -1.24$ (−1.99, −0.47)] and during 0 g [$t_{42.9} = -6.54$, $P < 0.001$, $d = -1.89$ (−2.84, −0.91)], and returned to baseline (pre-flight) in 1 g after P30 and post-flight (both *P*s > 0.3) (**Figure 3D**). Numerically, *Hit* and *False Alarm Rates* were also further impaired in 0 g compared to 1 g before P0 resulting in a significantly lower d' [$t_{42.9} = -2.23$, $P < 0.031$, $d = -0.64$ (−1.26, −0.01)]. Comparisons of all points in time of the different task variables are provided in **Supplementary Table 2**.

Factors Associated With the Experimental Set-Up of a Parabolic Flight Campaign

Motion Sickness

The MSSQ score of 5 ± 4.4 revealed that participants were less susceptible to intrinsic motion sickness than the general population (MSSQ percentile: 23 ± 18.4 vs. a norm of 50). One participant experienced discomfort during the flight after inflight data collection was completed. Due to continued discomfort after landing, post-flight data could not be collected for this participant.

Scopolamine

All participants received scopolamine in the morning prior to the flight. **Table 1** shows the parameters of CPT and PVT before and 30 min after subcutaneous injection of scopolamine. After scopolamine injection, RT was longer for the CPT and PVT, but only significant for the latter [$F_{1,11.8} = 8{,}31$, $P = 0.014$, $d = 0.83$ (0.16, 1.48)]. Numerically, a lower *False Alarm Rate* was observed after the medication for the CPT, but not the PVT. Longer RTs in the CPT were accompanied by a higher *Hit Rate* (both *P*s > 0.3) suggesting a strategy change. To control for a speed-accuracy tradeoff, we reanalyzed *Hit Rate* of CPT using RT as a covariate. After correction the improvement in *Hit Rate* from pre to post-medication was still discernable, though not significant ($P = 0.089$).

Stress

On average, cortisol levels increased from baseline throughout the parabolic flight reaching a peak after the 30th parabola before decreasing again at post-flight (effect of *Time*: $F_{5,48.3} = 2.61$, $P = 0.036$). Pre-planned contrasts revealed that cortisol concentrations measured after the last parabola (after P30) were significantly higher compared to baseline [$t_{48.3} = 3.14$, $P = 0.014$, $d = 0.91$ (0.21, 1.57)]. Visual inspection of the data revealed two types of responders, those whose cortisol peaked after the last parabola (High-P30), and those who reached their highest cortisol level before the first parabola (High-P0). **Figure 4** shows the time course of salivary cortisol concentrations for all subjects and for each subgroup. Compared to baseline, salivary cortisol levels of High-P30 increased significantly throughout the parabolic flight [P15: $t_{43.6} = 3.16$, $P = 0.009$, $d = 25.50$ (8.83, 41.62) and P30: $t_{43} = 8.05$, $P < 0.001$, $d = 25.18$ (8.71, 41.09)], and decreased post-flight. For High-P0, only slight changes in cortisol levels that were not significant were observed. Accordingly, group differences between High-P30 and High P-0 were observed at P15, P30, and post-flight [$t_{25.2} = -2{,}07$, $P = 0.049$, $d = 14.75$ (5.05, 24.11), $t_{22} = -5.68$, $P < 0.001$, $d = 12.88$ (4.39, 21.56), $t_{22.7} = -3.91$, $P < 0.001$, $d = 13.31$ (4.54, 22.03) respectively]. The changes in cortisol concentrations obtained prior to the first and after the last parabola were associated with self-reported sleep quality (Spearman's $\rho = 0.79$, $P = 0.012$), but not with total sleep duration ($\rho = 0.56$, $P = 0.117$).

Anxiety and Mood States

Participants were characterized by a low trait anxiety score of 34.2 ± 3.5 (range: 26–39) relative to the norm. State anxiety changed throughout the parabolic flight campaign, increasing from 25.9 ± 1.9 on the day of arrival (baseline) to 28.5 ± 1.9 on the morning before the flight, and decreasing below baseline to 23.8 ± 2.03 post-flight (effect of *Time*: $F_{2,\ 20.38} = 2.63$, $P = 0.097$). Pre-planned contrasts showed that the decrease in anxiety from pre-flight to post-flight was close to statistical significance [$t_{20.6} = 2.27$, $P = 0.068$, $d = 0.66$ (0.02, 1.27)]. Furthermore, lower scores in the subscales of tension-anxiety and anger-hostility of the POMS questionnaire were observed after

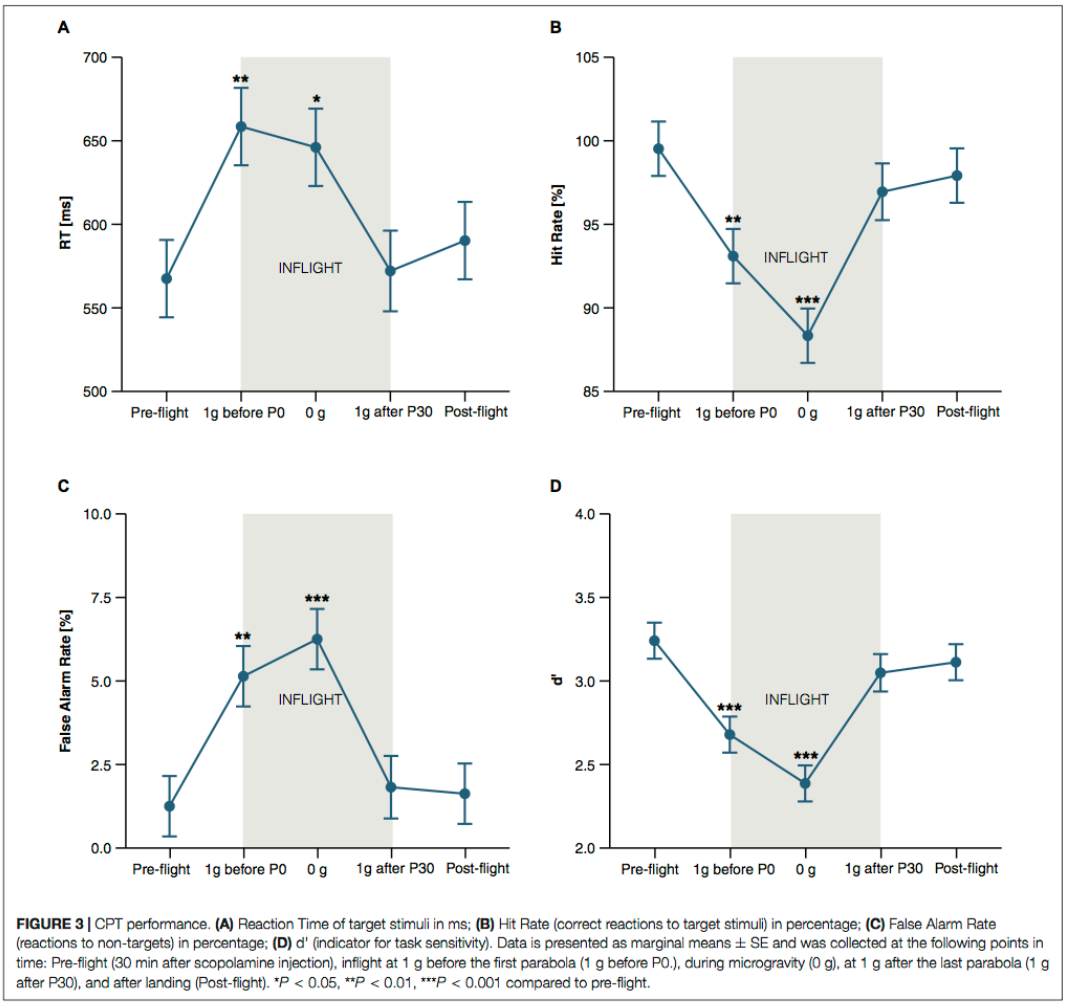

FIGURE 3 | CPT performance. **(A)** Reaction Time of target stimuli in ms; **(B)** Hit Rate (correct reactions to target stimuli) in percentage; **(C)** False Alarm Rate (reactions to non-targets) in percentage; **(D)** d' (indicator for task sensitivity). Data is presented as marginal means ± SE and was collected at the following points in time: Pre-flight (30 min after scopolamine injection), inflight at 1 g before the first parabola (1 g before P0.), during microgravity (0 g), at 1 g after the last parabola (1 g after P30), and after landing (Post-flight). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to pre-flight.

TABLE 1 | CPT and PVT performance before and after scopolamine injection*.

	Pre-medication	Post-medication	DF_1, DF_2	F	P
CPT					
RT (ms)	542 (21.1)	568 (21.1)	1, 11	0.91	0.360
Hit Rate (%)	99.0 (0.4)	99.5 (0.4)	1, 11	1.00	0.339
False Alarm Rate (%)	1.78 (0.5)	1.22 (0.5)	1, 22	0.58	0.455
PVT					
RT (ms)	307 (10.5)	339 (10.5)	1, 11.8	8.31	0.014
Hit Rate (%)	97.5 (1.46)	95.9 (1.46)	1, 11.6	1.81	0.204
False Alarm Rate (%)	na	na	na	na	na

Data represent marginal means and standard error in parentheses. CPT, Continuous Performance Task; PVT, Psychomotor Vigilance Task; RT, Reaction Time in ms; Hit Rate, correct reaction to target stimuli in percentage; False Alarm Rate, reaction to non-targets in percentage; DF_1, numerator degrees of freedom; DF_2, denominator degrees of freedom; F, F-statistics; P, p-value. Data were obtained before and after 30 min of a subcutaneous scopolamine injection of 0.175 mg.

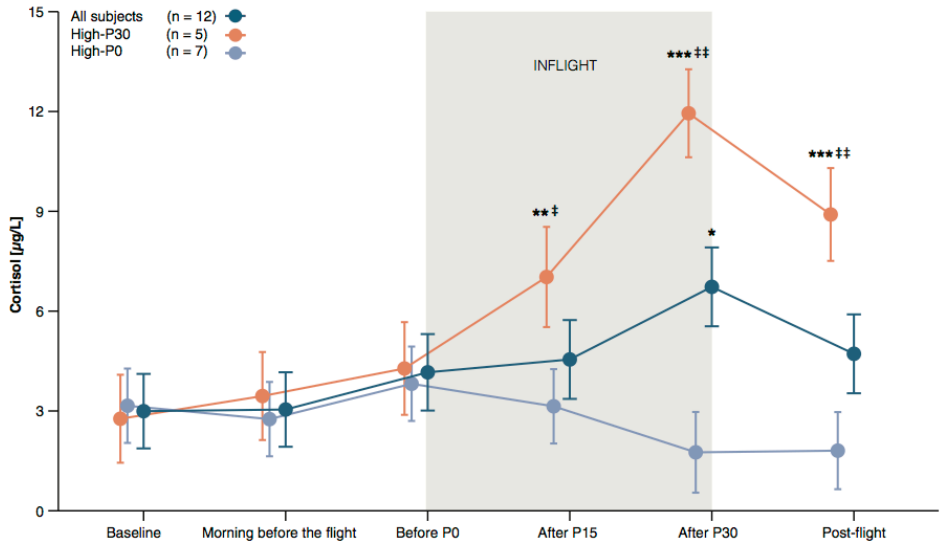

FIGURE 4 | Time course of salivary cortisol levels. Baseline saliva was collected between 9 am and noon on the day of arrival. Two different patterns could be observed, with the highest measured cortisol either after P30 (High-P30) or before P0 (High-P0). Orange line represents participants of High-P30, violet line represents participants of High-P0, dark blue line shows data of all participants. Data are presented as marginal means ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to baseline, ‡$P < 0.05$, ‡‡$P < 0.001$ compared to High-P0.

parabolic flight exposure [$F_{1,11} = 5.62$, $P = 0.037$, $d = -0.68$ (-1.3, -0.04) and $F_{1,11} = 9.67$, $P = 0.010$, $d = -0.9$ (-1.56, -0.21) respectively]. Total mood disturbance (TMD) decreased from 0.96 ± 4.49 to −6.13 ± 4.49, nearly reaching statistical significance [$F_{1,11} = 4.46$, $P = 0.058$, $d = -0.61$ (-1.22, 0.02)]. There were no significant changes in the scores of depression, vigor, fatigue, and confusion (all $Ps > 0.23$). **Table 2** shows a detailed overview of participants' mood states before and after the flight.

Sleep Quality

The participants slept approximately 6h20min ± 1 h (range between 5 and 8 h) the night before the parabolic flight with self-reported sleep quality of 7.4 ± 1.7 (visual analog scale between 0 and 10 with 10 indicating the highest sleep quality). Furthermore, participants reported that they woke up approximately once during the night, and having a generally good state of mind (visual analog scale: 8.3 ± 1.3).

Correlation Between Cognitive Performance, Cortisol, and Emotional States

A similar time course of participant's salivary cortisol levels and state anxiety was observed compared to the time course of CPT performance parameters. Therefore, we investigated the relationships between state anxiety, cortisol, and mood states (Anger, Tension, and TMD) with participant's CPT performance

TABLE 2 | Participants' Profile of Mood States (POMS) before and after the parabolic flight*.

	Pre-flight	Post-flight	$F_{1,11}$	P	Effect size (95% CI)
Tension	5.83 (1.14)	3.46 (1.14)	5.62	0.037	–0.68 (–1.3, –0.04)
Depression	1.92 (0.56)	1.67 (0.56)	0.20	0.667	–0.13 (–0.69, 0.44)
Anger	5.42 (1.04)	2.04 (1.04)	9.67	0.010	–0.9 (–1.56, –0.21)
Vigor	20.90 (1.02)	22.20 (1.02)	1.56	0.238	0.36 (–0.23, 0.94)
Fatigue	4.37 (1.35)	5.44 (1.35)	0.66	0.432	0.24 (–0.34, 0.8)
Confusion	4.33 (1.27)	3.42 (1.27)	1.61	0.231	–0.37 (–0.94, 0.23)
TMD	0.96 (4.49)	–6.13 (4.49)	4.46	0.058	–0.61 (–1.22, 0.02)

Data represent marginal means and standard error in parentheses. TMD, Total Mood Disturbance, F, F-statistics; P, p-value; Effect size is Cohen's d in 95% confidence intervals (95% CI).

using repeated measures correlation. A higher level of anxiety was associated moderately with slower *Reaction Time* ($r = 0.52$, $P = 0.019$), lower *Hit Rate* ($r = -0.4$, $P = 0.06$), and higher *False Alarm Rate* ($r = 0.60$, $P = 0.005$). We did not find any significant correlation between cortisol concentrations and CPT parameters (all *P*s > 0.182) and between mood states and CPT (all *P*s > 0.407).

DISCUSSION

For further space missions, it is important to identify the neurobehavioral implications of weightlessness and transitions between gravity levels. Parabolic flight maneuvers provide a unique opportunity to assess the acute effects of hyper- and hypogravity on cognitive performance. Stahn et al. (2020) and others have shown that spatial cognition is significantly impaired during altered gravity conditions (Grabherr et al., 2007; Grabherr and Mast, 2010; Clément et al., 2016). It is unclear to what extend these effects are also observed for other cognitive domains as previous studies have also reported improvements in tasks targeting executive functions (Wollseiffen et al., 2016, 2019).

Here, we investigated the effects of microgravity during parabolic flights on a Go/No-Go Continuous Performance Task. We also aimed to identify potential confounders associated with the experimental setting of parabolic flight experiments. We observed a deterioration in performance of the CPT for both conditions, i.e., before experiencing the first parabola and during the microgravity phase compared to pre-flight testing on-ground characterized by a lower *Hit Rate* and increased *False Alarm Rate* and *Reaction Time*. The performance impairments observed during the flight are likely to be related to various factors associated with the anticipation of the first parabola experience. For instance, compared to the day of arrival (baseline), an increased level of anxiety was reported by the participants immediately before take-off that decreased below baseline post-flight. The changes in anxiety were moderately associated with the changes in *Reaction Time*, *Hit* and *False Alarm Rate* of the CPT.

Additional aspects that may have impinged the attentional capacity on-board are the unfamiliar workload associated with the preparation of the experiment, and alternating the attentional focus between the experiment, pilot announcement, and directions given by the operators and safety crew. The impairments in CPT performance variables observed during 1 g prior to the first parabola were further deteriorated during 0 g, reflecting a gravity effect on attentional processing. We suggest that changes in sensory perception of the own body and in the control of movements in microgravity combined with the emotional state increase the demand of divided attention. All participants were novices to the microgravity experience. Experiencing weightlessness and responding to this novel posture can have considerable impact on the attentional load. The parietotemporal sensory cortex, precuneus, hippocampus as well as subcortical structures such as the thalamus integrate information from the somesthetic, visual, and the vestibular system that support spatial abilities (Besnard et al., 2015; Smith, 2017) including self-perception and one's position during locomotion, the perception of verticality, mental rotation, orientation, navigation, and spatial memory (Lopez, 2016). The vestibular system is the sensor of terrestrial gravity by its otolithic component and plays a key role in the cortical calibration of visual and somesthetic information related to spatial orientation (Cullen, 2012). The otolithic responses of the vestibular organ are inhibited in weightlessness (Probst et al., 1996; Reschke et al., 2018) and spatial abilities are impaired during hyper- and hypogravity (Mittelstaedt and Glasauer, 1993a,b; Grabherr and Mast, 2010; Stahn et al., 2020). This notion is also supported by a recent functional imaging study, reporting decreases of intrinsic connectivity within the right temporoparietal junction in first-time flyers after parabolic flight (Van Ombergen et al., 2017). Together, these data suggest that vestibular sensory awareness in microgravity phases may play a role in increasing attentional loading on spatial cognitive functions during microgravity phases in which participants are "spatially lost." Thus, prioritizing self-perception and balance control during microgravity may challenge spatial cognition. The direct effect of the vestibular system on attention remains poorly investigated. It has been shown that vestibular deficiency impaired attention abilities in rodents (Zheng et al., 2009) and humans (Bigelow and Agrawal, 2015) including attention related to visual reward-seeking (Blini et al., 2018). It can be speculated that the decrease in sustained attention for a specific task in microgravity phases is somewhat related to disturbances of the vestibular input and its associated changes in spatial cognition requiring divided attention.

Likewise, the effects of the somesthetic system on attention are currently not well understood. The participants remained secured in a controlled free-floating position that is expected to decrease the somesthetic effect on attention compared to unrestricted free-floating. Additionally, it can also be presumed that the emotional states associated with the sensory perception and the stress-related hormonal effects modulate attention and need to be considered as confounders of attentional control during parabolic flight. The interactions between emotion and attention are well documented (Schultebraucks et al., 2016; Dolcos et al., 2019). Several studies support the role of vestibular inputs for emotional processing (Lopez, 2016; Rajagopalan et al., 2017; Barona-de-Guzmán et al., 2018), including the fear of falling (Schlick et al., 2016) or panic disorders (Perna et al., 2001). It would be worthwhile to further evaluate the attentional abilities of participants with considerable previous parabolic flight experiences. This may allow to discriminate the effects between an acute and an adaptive effect of this particular environment. We expect that a significant history of parabolic flight experience will attenuate the performance decline observed prior to the first parabola. This hypothesis is based on the assumption that frequent flyers are less prone to motion sickness (Golding et al., 2017) and other factors associated with the parabolic flight environment. It is very likely that data of participants with previous flight experience would provide more robust measures of neurobehavioral performance because some of the potential confounding effects are minimized due to the familiarity with the experimental setting and reduced novelty of the g-transitions. In contrast to this hypothesis, Wollseiffen et al. (2016) did not find any differences between

experienced and first-time flyers in a complex arithmetic task. They also reported faster reaction times for the highest level of difficulty in the arithmetic task in 0 g compared to 1 g. They attributed the improved performance to the microgravity-induced increases in cerebral blood flow and oxygenation (Blaber et al., 2013; Wollseiffen et al., 2019). Notably, the participants' responses were also less accurate in 0 g, suggesting a change in response strategy. Thus, it cannot be concluded *per se* that increased cerebral blood flow increases cognitive performance. It can be rather assumed that faster reaction times during altered gravity levels in parabolic flight may also be associated with the experimental conditions of parabolic flight studies, including, but not limited to, performing tasks during bouts of 20 s of weightlessness under considerable time constraints. Additionally, response speed may also vary throughout the flight, independent of the gravity level as observed in the present study.

We also investigated the emotional state using surveys and determined salivary cortisol as an indicator of stress level. Cortisol plays an important role in various physiological processes and is an acceptable marker for stress (Hannibal and Bishop, 2014). The highest cortisol concentration was observed at the end of the parabolic flight. This effect was also reported in previous parabolic flights using serum samples (Schneider et al., 2009). Inspection of individual responses revealed two different phenotypes: one cluster of participants showed their highest cortisol concentrations before the flight that decreased after the last parabola (around noon), and a second cluster those showed the highest cortisol levels after the last parabola. Peak cortisol production usually occurs in the early morning and declines throughout the day with lowest cortisol levels in the late evening and first half of the night (Tsigos and Chrousos, 2002). We conclude that participants showing a decreasing pattern in cortisol levels throughout the flight are less stressed because of the typical circadian pattern of cortisol secretion. In contrast, participants whose cortisol secretion peaked around noon seemed to be more stressed. Additionally, we also observed a strong correlation between self-reported sleep quality and higher cortisol secretion during the flight. However, it is unclear whether the circadian pattern of cortisol secretion was disrupted due to reduced sleep quality, or whether perceived stress was the cause of poor sleep. In the present study, we did not find an association between cortisol concentrations and sleep duration, self-reported anxiety or attention. It is possible that self-reported data on anxiety may be confounded by a response bias such as social desirability and acquiescent in the present cohort, so that the recorded anxiety levels may not have reliably reflected the participants' true affective states (Kreitchmann et al., 2019). The present data on self-reported anxiety should therefore be interpreted cautiously. We also acknowledge that four out of five participants who showed their cortisol maximum at noon flew on the first day of the campaign. Thus, they may have been more stressed relative to the participants flying on the second or third day of the parabolic flight campaign.

According to the questionnaires, all participants showed an increase in anxiety in the morning of the flight that decreased after the flight. Additionally, we also noted that participants had significantly higher ratings for the subscales "Tension" and "Anger" before the parabolic flight, suggesting an increased arousal and nervousness associated with the uncertainties of the parabolic flight experience. Increases in cortisol levels and self-reported arousal indicate an activation of the sympathetic system (Thau and Sharma, 2019) that may not have been only induced by the novel parabolic flight experience itself, but also by the cognitive task the participants had to complete. Indeed, mental challenges such as mental arithmetic have shown to increase heart rate during different g-levels by 16–18% (Osborne et al., 2014), delaying (pre)syncopes that may occur in consequence of the downward fluid shifts during the transition from hypo- to hypergravity (Goswami et al., 2012; Blaber et al., 2013). Whether the paradigm employed in this study has also substantially activated the sympathetic nervous system cannot be exclusively determined because cardiovascular data were not collected.

It is well established that sleep deprivation reduces alertness and level of attention (Lim and Dinges, 2008; Maire et al., 2018). We noted that the sleep duration and perceived sleep quality of the night before the parabolic flight were reasonable with one wake-up on average. The effect of sleep debt that we expected prior to the experiment remained moderately. However, the effect of sleep during the nights prior to the parabolic flight experiment remains to be confirmed by quantitative measurements such as actigraphy, overnight echocardiography or electroencephalography. To the best of our knowledge, no studies on sleep debt related to attention and cognition in parabolic flights have been published previously.

Our participants presented a low sensitivity level of intrinsic motion sickness susceptibility, a low level of trait anxiety, and volunteered for a parabolic flight, making a natural "selection" of participants (Collado et al., 2014, 2018; Montag et al., 2016). A lower motion sickness susceptibility of parabolic flight participants compared to control subjects was already reported in a previous study by Golding et al. (2017). However, all participants of the present study agreed to be preventively treated for motion sickness at a dose of 0.7 mL, i.e., 0.175 mg, of scopolamine that was administered subcutaneously. Only a single participant felt moderately sick during the flight after inflight data collection had been completed. To evaluate performance degradations in response to scopolamine side effects such as reduced arousal and fatigue, we assessed participant's vigilance and sustained attention. Thirty minutes after scopolamine injection, we observed significant slower RT of the PVT, whereas the RT of the CPT only tended to increase. Previous studies have investigated the effects of scopolamine under laboratory conditions, excluding parabolic flight. Rusted and Warburton (1988) reported cognitive impairments at a dose of 0.6 mg scopolamine on problem-solving, visuospatial abilities, and spatial memory. This has also been confirmed by studies of Ebert et al. (1998) and Fredrickson et al. (2008) where different increments of scopolamine doses from placebo up to 0.8 mg impinged psychomotor function and reaction time, visual learning, executive function, and working memory. Decreases in cognitive performance peaked after one to two hours after drug injection but were still observed up to 8 h later.

Flicker et al. (1990) also confirmed that high doses (0.44 and 0.63 mg) affected the performance of verbal and visuospatial recall, visual recognition memory, visuospatial praxis, visual-perceptual function, and psychomotor speed. Lower doses of 0.22 mg induced only peripheral signs, but did not impair cognitive functions (Flicker et al., 1990). Similarly, Bestaven and colleagues reported no effect on reaction time in a scoring task up to 30 min after the injection of scopolamine at a dose of 0.2 mg (Bestaven et al., 2016). However, these authors reported an effect on posture and on vestibulomotor control of the lower limbs (Bestaven et al., 2016), which could be related to the effect of scopolamine on the brainstem reported in animals (Gall et al., 2007). We found slower RT in CPT and PVT, whereas only the latter reached the level of statistical significance. Increases in RT of CPT were related to a higher *Hit Rate*, suggesting a speed-accuracy tradeoff after scopolamine administration. The differences between the PVT and CPT performance in response to the scopolamine could also be related to the high sensitivity of the PVT to wakefulness (Lim and Dinges, 2008). However, we also critically acknowledge that this discrepancy may also be the result of data acquisition under different experimental conditions using different technologies that were chosen due to time and hardware constraints. The CPT was performed in a controlled free-floating supine position with an immersive setup that minimized external visual distractors on a 15-in screen, whereas the PVT was performed in a seated position on a wrist-worn diving computer equipped with a 2-in display. Because of these considerable technical and methodological differences, the comparison of these data warrants some caution.

CONCLUSION

Taken together, our data show that the experimental setting of the parabolic flight results in a significant performance decline in a Go/No-Go Continuous Performance Task, which is further aggravated during weightlessness. We attribute these findings to increased stress and anxiety state prior to the flight, and altered vestibular input related to cognitive functions including self-perception and spatial orientation during the different gravity conditions in-flight. Anti-motion sickness medication with a low dose of subcutaneous scopolamine affected slightly RT of the PVT, but not CPT. Our results indicate that it is important to control for contributing factors such as participants' emotional state, sleep quality, and medication when designing behavioral research for parabolic flight experiments. Additionally, the control condition of 1 g should be administered as time coherent as possible with hypo- and hypergravity conditions as the impact of the contributing factors can vary throughout the flight and experiment. Future studies in larger samples are needed to verify whether the observed effects are limited to first-time flyers and to investigate potential sex-specific differences.

DATA AVAILABILITY STATEMENT

The acceleration and behavioral data that support the findings of this study are freely available on the Open Science Framework (OSF) (https://osf.io/kxn9v/).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes Nord Ouest III, Caen, France. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AF-W and SB wrote the manuscript. M-LM, SB, and CB designed the experiment. YL was in charge of the rack and technical set up. M-LM, CB, and BP performed the data collection. AF-W performed the statistical analyses. MH coordinated, supervised, and financed saliva processing. ACS, KB, and CB revised the manuscript. All authors have discussed and interpreted the results and contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.675426/full#supplementary-material

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Supplementary Materials:

Impaired attentional processing during parabolic flights

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Supplementary Tables

Table S1. Acceleration data of the hypogravity phases for parabolas 11 to 15 and 26 to 30 for each flight day and for each axis respectively.

	Flight day I			Flight day II			Flight day III		
	x-axis	y-axis	z-axis	x-axis	y-axis	z-axis	x-axis	y-axis	z-axis
P11	-0.013 (-0.037 – 0.012)	-0.004 (-0.014 – 0.006)	0.006 (-0.022 – 0.037)	-0.010 (-0.035 – 0.020)	-0.004 (-0.014 – 0.006)	0.002 (-0.029 – 0.078)	-0.010 (-0.031 – 0.010)	-0.005 (-0.016 – 0.002)	0.003 (-0.023 – 0.090)
P12	-0.011 (-0.035 – 0.008)	-0.004 (-0.020 – 0.006	0.006 (-0.023 – 0.094)	-0.011 (-0.037 – 0.016)	-0.005 (-0.016 – 0.008)	0.008 (-0.014 – 0.065)	-0.010 (-0.031 – 0.012)	-0.005 (-0.018 – 0.002)	0.008 (-0.016 – 0.096)
P13	-0.010 (-0.031 – 0.013)	-0.003 (-0.012 – 0.004)	0.009 (-0.006 – 0.074)	-0.010 (-0.031 – 0.016)	-0.004 (-0.014 – 0.006)	-0.005 (-0.027 – 0.078)	-0.011 (-0.035 – 0.012)	-0.005 (-0.016 – 0.002)	0.010 (-0.012 – 0.084)
P14	-0.010 (-0.035 – 0.014)	-0.004 (-0.016 – 0.002)	0.005 (-0.012 – 0.090)	-0.009 (-0.037 – 0.016)	-0.004 (-0.010 – 0.002)	0.006 (-0.004 – 0.080)	-0.010 (-0.031 – 0.008)	-0.005 (-0.022 – 0.008)	0.004 (-0.014 – 0.084)
P15	-0.009 (-0.033 – 0.016)	-0.004 (-0.020 – 0.010)	0.004 (-0.012 – 0.096)	-0.008 (-0.027 – 0.016)	-0.005 (-0.010 – 0.000)	0.000 (-0.027 – 0.078)	-0.009 (-0.029 – 0.020)	-0.004 (-0.021 – 0.006)	0.009 (-0.010 – 0.066)
P26	-0.011 (-0.039 – 0.012)	-0.003 (-0.018 – 0.012)	-0.002 (-0.027 – 0.059)	-0.009 (-0.027 – 0.016)	-0.003 (-0.012 – 0.006)	0.009 (-0.010 – 0.074)	-0.008 (-0.037 – 0.022)	-0.003 (-0.010 – 0.008)	0.002 (-0.023 – 0.082)
P27	-0.011 (-0.035 – 0.012)	-0.005 (-0.018 – 0.006)	0.001 (-0.012 – 0.059)	-0.009 (-0.033 – 0.012)	-0.003 (-0.014 – 0.006)	0.006 (-0.016 – 0.082)	-0.009 (-0.031 – 0.020)	-0.002 (-0.014 – 0.012)	0.004 (-0.014 – 0.088)
P28	-0.006 (-0.027 – 0.022)	-0.002 (-0.006 – 0.006)	0.007 (-0.025 – 0.074)	-0.009 (-0.033 – 0.016)	-0.003 (-0.010 – 0.004)	0.004 (-0.021 – 0.084)	-0.009 (-0.035 – 0.018)	-0.002 (-0.010 – 0.006)	0.005 (-0.008 – 0.059)
P29	-0.010 (-0.031 – 0.016)	0.000 (-0.018 – 0.014)	0.006 (-0.010 – 0.090)	-0.010 (-0.037 – 0.018)	-0.004 (-0.012 – 0.006)	0.008 (-0.018 – 0.082)	-0.008 (-0.029 – 0.018)	-0.002 (-0.012 – 0.004)	0.005 (-0.014 – 0.066)
P30	-0.007 (-0.027 – 0.018)	-0.004 (-0.014 – 0.008)	0.001 (-0.033 – 0.078)	-0.010 (-0.033 – 0.018)	-0.004 (-0.010 – 0.004)	-0.006 (-0.039 – 0.080)	-0.009 (-0.029 – 0.014)	-0.003 (-0.012 – 0.004)	0.007 (-0.012 – 0.094)

P, number of parabola; x-axis, Gx acceleration from tail to front of the aircraft; y-axis, Gy acceleration from left to right wings; z-axis, Gz acceleration from floor to ceiling. Data is presented as mean (averaged over 22 seconds) and range (minimum – maximum) for each parabola when participants completed a continuous performance task.

Table S2. Contrasts comparing the different points in time (Pre-flight, 1 g before P0, 0 g, 1 g after P30, and Post-flight) on CPT performance characteristics (Reaction Time, Hit Rate, False Alarm Rate, d').

Contrast	Variable	Estimate	SE	DF	*t*	*P*	Effect Size (95% CI)
1 g before P0 - Pre-flight	RT	91.00	30.5	43.2	2.98	0.019	0.86 (0.18, 1.52)
	Hits	-6.43	2.2	43.1	-2.93	0.016	-0.85 (-1.50, -0.17)
	FA	4.49	1.2	42.9	3.66	0.002	1.06 (0.33, 1.76)
	d'	-0.57	0.1	42.9	-4.31	<0.001	-1.25 (-1.99, -0.47)
0 g - Pre-flight	RT	78.58	30.5	43.2	2.58	0.040	0.74 (0.09, 1.37)
	Hits	-11.19	2.2	43.1	-5.11	<0.001	-1.47 (-2.29, -0.63)
	FA	5.77	1.2	42.9	4.71	<0.001	1.36 (0.55, 2.14)
	d'	-0.87	0.1	42.9	-6.54	<0.001	-1.89 (-2.84, -0.91)
1 g after P30 - Pre-flight	RT	4.57	31.2	43.7	0.15	0.919	0.04 (-0.52, 0.61)
	Hits	-2.58	2.2	43.7	-1.15	0.514	-0.33 (-0.91, 0.26)
	FA	0.66	1.3	43.3	0.52	1.000	0.15 (-0.42, 0.72)
	d'	-0.19	0.1	43.3	-1.43	0.321	-0.41 (-0.99, 0.19)
Post-flight - Pre-flight	RT	22.75	30.5	43.2	0.75	0.919	0.22 (-0.36, 0.78)
	Hits	-1.61	-2.5	43.1	-0.73	0.514	-0.21 (-0.78, 0.37)
	FA	0.43	1.2	42.9	0.35	1.000	0.10 (-0.47, 0.67)
	d'	-0.13	0.1	42.9	-0.98	0.332	-0.28 (-0.85, 0.30)
0 g - 1 g before P0	RT	12.42	30.5	43.2	-0.41	0.686	-0.12 (-0.68, 0.45)
	Hits	-4.76	2.2	43.1	-2.17	0.099	-0.63 (-1.24, 0.01)
	FA	1.28	1.2	43.3	1.05	0.301	0.30 (-0.28, 0.87)
	d'	-0.29	0.1	42.9	-2.24	0.030	-0.65 (-1.26, -0.01)
1 g after P30 - 1 g before P0	RT	-86.43	31.2	43.7	-2.77	0.025	-0.80 (-1.44, -0.13)
	Hits	3.85	2.2	43.7	1.72	0.099	0.50 (-0.12, 1.09)
	FA	-3.83	1.3	43.3	-3.05	0.008	-0.88 (-1.54, -0.19)
	d'	0.37	0.1	43.3	2.78	0.017	0.80 (0.13, 1.44)
Post-flight - 1 g before P0	RT	-68.25	30.5	43.2	-2.24	0.061	-0.65 (-1.26, -0.01)
	Hits	4.82	2.2	43.1	2.20	0.099	0.64 (0.00, 1.25)
	FA	-4.05	1.2	42.9	-3.31	0.006	-0.96 (-1.63, -0,25)
	d'	0.43	0.1	42.9	3.32	0.006	0.96 (0.25, 1.63)
1 g after P30 - 0 g	RT	-74.01	31.2	43.7	-2.37	0.067	-0.68 (-1.30, -0.04)
	Hits	8.61	2.2	43.7	3.84	<0.001	1.11 (0.37, 1.82)
	FA	-5.11	1.3	43.3	-4.07	<0.001	-1.17 (-1.90, -0.41)
	d'	0.66	0.1	43.3	4.95	<0.001	1.43 (0.60, 2.23)
Post-flight - 0 g	RT	-55.83	30.5	43.2	-1.83	0.148	-0.53 (-1.12, 0.09)
	Hits	9.58	2.2	43.1	4.37	<0.001	1.26 (0.48, 2.02)
	FA	-5.34	1.2	42.9	-4.35	<0.001	-1.26 (-2.01, -0.47)
	d'	0.73	0.1	42.9	5.56	<0.001	1.61 (0.72, 2.46)
Post-flight - 1 g after P30	RT	18.18	31.2	43.7	0.58	1.000	0.17 (-0.41, 0.73)
	Hits	0.97	2.2	43.7	0.43	0.668	0.12 (-0.45, 0.69)
	FA	-0.22	1.3	43.3	-0.18	1.000	-0.05 (-0.62, 0.52)
	d'	0.06	0.1	43.3	0.47	0.638	0.14 (-0.43, 0.70)

RT, Reaction Time of target stimuli in ms; Hits, Hit Rate (correct reactions to target stimuli) in percentage; FA, False Alarm Rate (reactions to non-targets) in percentage; d', indicator for task sensitivity. n = 12. *df*, degrees of freedom, *t*, t-ratio; *P*, p-value; Effect size is Cohen's *d* and the corresponding 95% confidence interval (95% CI). Data is presented as marginal means ± SE

and was collected at the following points in time: Pre-flight (30 min after scopolamine injection), inflight at 1 g before the first parabola (1 g before P0), during microgravity (0 g), at 1 g after the last parabola (1 g after P30), and after landing (Post-flight).

Curriculum Vitae

My curriculum vitae will not be published in the eletronic version of the work for data protection reasons.

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List of Publications and Scientific Communications

Publications:

Brauns, K., **Friedl-Werner, A.**, Gunga, H.-C., & Stahn, A. C. (2021). Effects of two months of bed rest and antioxidant supplementation on attentional processing. *Cortex*, *141*, 81–93.

IF: 4.027

Friedl-Werner, A., Machado, M.-L., Balestra, C., Liegard, Y., Philoxene, B., Brauns, K., Stahn, A. C., Hitier, M., & Besnard, S. (2021). Impaired Attentional Processing During Parabolic Flight. *Frontiers in Physiology*, *12*, 675426.

IF: 4.566

Mendt, S., Brauns, K., **Friedl-Werner, A.,** Belavy, D. L., Steinach, M., Schlabs, T., Werner, A., Gunga, H.-C., & Stahn, A. C. (2021). Long-Term Bed Rest Delays the Circadian Phase of Core Body Temperature. *Frontiers in Physiology*, *12*, 658707.

IF: 4.566

Brauns, K., **Friedl-Werner, A.**, Maggioni, M. A., Gunga, H. C., & Stahn, A. C. (2021). Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity. *Frontiers in Physiology*, *12*.

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IF: 6.566

Stahn, A. C., Riemer, M., Wolbers, T., **Werner, A.**, Brauns, K., Besnard, S., Denise, P., Kühn, S., & Gunga, H.-C. (2020). Spatial Updating Depends on Gravity. *Frontiers in Neural Circuits*, *14*.

IF: 3.492

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IF: 3.201

Masatli, Z., Nordine, M., Maggioni, M. A., Mendt, S., Hilmer, B., Brauns, K., **Werner, A.**, Schwarz, A., Habazettl, H., Gunga, H.-C., & Opatz, O. S. (2018). Gender-specific cardiovascular reactions to +GZ interval training on a short arm human centrifuge. *Frontiers in Physiology*, *9*.

IF: 3.201

Kühn, S., **Werner, A**., Lindenberger, U., & Verrel, J. (2014). Acute immobilization facilitates premotor preparatory activity for the non-restrained hand when facing grasp affordances. *NeuroImage, 92,* 69-73.

IF: 6.357

Scientific Communications:

Werner, A., Brauns, K., Gunga, H.-C., Kühn, S., Stahn, A. C.: Changes in neuronal activity and episodic memory after 30 days of isolation and confinement. Presented at the *39th International Society for Gravitational Physiology & European Space Agency Life Science Meeting* from 18 – 22 June 2018, Noordwijk, Netherlands.

Werner, A., Brauns, K., Gunga, H.-C., Kühn, S., Stahn, A. C.: Exercise as a countermeasure for impaired brain function? - Evidence from the RSL Bed Rest Study. Presented at the *39th International Society for Gravitational Physiology & European Space Agency Life Science Meeting* from 18 – 22 June 2018, Noordwijk, Netherlands.

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